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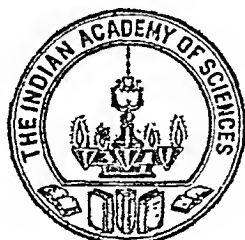
SECTION B

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# MORPHOLOGICAL STUDIES IN THE GRAMINEAE

## V. Vascular Anatomy of the Spike and Spikelets in the Andropogoneae\*

BY NARESH CHANDRA AND N. P. SAXENA

(School of Plant Morphology, Meerut)

Received August 7, 1963

(Communicated by Dr. V. Puri, F.A.Sc.)

### INTRODUCTION

THE tribe andropogoneae is a most complex and highly specialized tribe of the Gramineae and stands next only to Maydeae in the evolutionary sequence. The spikelets are binate at each node of the rachis—one sessile and perfect and the other pedicelled either fertile or reduced. Each spikelet has two outer empty glumes and two florets—the lower generally reduced in development and the upper generally perfect. No detailed study, however, seems to have been made of the spikelet anatomy of this tribe. Keng (1939) described in detail the gross morphology of the tribe. Long (1930) studied the vascular anatomy of the spike and spikelets of *Sorghum sudanense* and *S. halepense* and Arber (1934) has made a study of *Ischaemum rugosum* and certain other related grasses. This study was undertaken with a view to have a better understanding of the spikelet in the Gramineae in general. The present paper deals with the structure and vascular supply of twenty species belonging to five sub-tribes of Andropogoneae.

### MATERIAL AND METHOD

The following is the list of species included in this study.

#### Sub-tribe: SACCHARINAE

*Saccharum officinarum* Linn.

*Saccharum spontaneum* Linn.

*Erianthus munja* (Roxb.) Jeswiet

*Erianthus fulvus* Nees

*Imperata cylindrica* (Linn.) P. Beauv.

\* Research Contribution No. 56 from the School of Plant Morphology, Meerut College, Meerut.



## Sub-tribe ISCHIMINAE

*Ischaemum rugosum* Salisb

*Apluda mutica* Linn var *aristata* (Linn) Pilger

## Sub-tribe ROTTBOELLINAE

*Hemarthria compressa* (Linn f) R Rr

*Rottboellia exaltata* Linn f

*Ophiuros exaltatus* (Linn) Ktze

*Mnesithea laevis* (Rctr) Kunth

## Sub-tribe SORGHINAE

*Sorghum halepense* (Linn) Pers

*Vetiveria zizanioides* (Linn) Nash

## Sub-tribe ANDROPOGONINAE

*Dichanthium annulatum* (Forsk.) Stapf

*Bothriochloa pertusa* (Linn) Camus

*Arihaxon ciliaris* Beauv

*Cymbopogon martinii* (Roxb) Wats

*Heteropogon contortus* (Linn) Beauv ex R and S *Themeda* sp

*Themeda* sp

*Iselema antheophoroides* Hack

Most of the grasses were collected locally. The material of *Ischaemum rugosum*, *Themeda* sp and *Iselema antheophoroides* was collected from Lonavla and that of *Cymbopogon martinii* from Bangalore. Herbarium material of *Ophiuros exaltatus* was obtained through the courtesy of the Regional Botanist Botanical Survey of India Poona. The material of *Arihaxon ciliaris* and *Erianthus fulvus* was collected from Simla.

Spikes and spikelets of the various grasses were fixed in F.A.A. Some of the material required pretreatment with 5% KOH before dehydration. Customary methods of dehydration, clearing and embedding were followed. Sections were cut 10-14  $\mu$  thick and stained with Crystal violet—Erythrosin or Safranin—Fast green combination.

## *Morphological Studies in the Gramineae—I*

### — OBSERVATIONS —

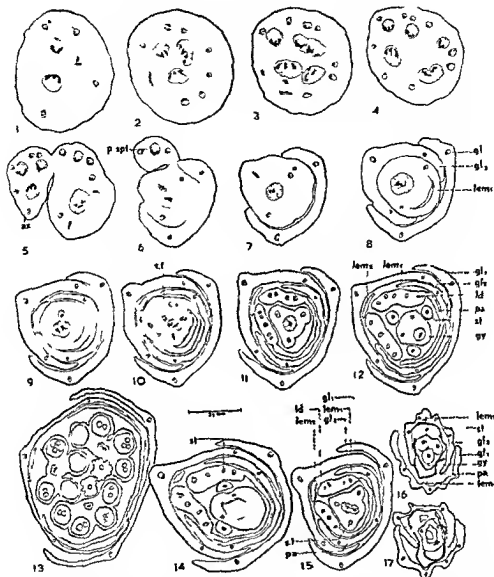
#### Sub-tribe: SACCHARINAE

The inflorescence is a large terminal panicle of racemes and is covered with soft delicate hairs. At each joint of a raceme, there is one sessile and one pedicelled spikelet. Each sessile spikelet has an outer glume, an inner glume, two lemmas—the outer generally empty while the inner one is paleate with a perfect bisexual flower, having two lodicules, three stamens and a gynoeceum with two plumose stigmas. Lodicules are, however, absent in *Imperata cylindrica*. The pedicelled spikelet is just similar to the sessile one.

In a transverse section of the axis there are three large central bundles and a few small bundles on the periphery (Fig. 1). All these bundles multiply at the base of a joint (Figs. 2-4). One large bundle and two small, one on either side of it pass out for the sessile spikelet and three similar bundles for the pedicelled spikelet (Fig. 5). In the sessile spikelet the two side bundles supply the outer empty glume while the central one expands out to form a mass of vascular tissue (Figs. 5 and 6). This gives out on either side one trace which divides into two each. These are the marginal nerves for the second empty glume and the lemma I (Figs. 6-8). The second empty glume also receives one median nerve from the central mass of vascular tissue (Fig. 7). Lemma II and its palea are hyaline non-vascular structures (Figs. 9 and 10). The central vascular mass now gives off two traces anterolaterally for the two lodicules which divide and subdivide immediately after separation from the central vascular mass (Figs. 9 and 10).

The remaining vascular tissue after supplying one trace to each of the three stamens enters the base of the gynoeceum (Fig. 11). It gives off two traces laterally for the ovary wall and itself supplies the solitary ovule attached to the posterior side of the gynoeceum (Fig. 12). The two bundles traversing the ovary wall pass out into the stigmas (Fig. 13). In one case in *S. officinarum* there were only two lateral stamens (Fig. 14). The pedicelled spikelet is also similar to the sessile one in its vasculature (Fig. 15).

In *Saccharum spontaneum*, the lemma I has only one median nerve. In *Erianthus munja* and *E. fulvus* the outer empty glume is 4 nerved and lemma I and lemma II have a single median nerve. In *Imperata cylindrica* the two outer glumes are 4-nerved each while the two lemmas and the palea of lemma II are non-vascular. There are only two stamens which are displaced towards the anterior side (Figs. 16 and 17). In all these grasses the pedicelled spikelet resembles the sessile one in its vascular anatomy.



FIGS. 1-17 Figs. 1-15 *Saccharum officinarum*.—Figs. 1-6 Serial transverse sections of the rachis from base upwards Figs 7-13 Serial transverse sections of the sessile spikelet from base upwards. Fig. 14 T.S Abnormal spikelet showing two stamens Fig. 15 TS pedicelled spikelet. Figs. 16 and 17 *Imperata cylindrica* transverse sections of the sessile spikelet. (ax, axis, gl, glume; gy, gynoeccium, ld, lodicule, lem, lemma; pa, palea, p spt, pedicelled spikelet, st, stamen, st, stamen-trace)

#### Sub-tribe: ISCHIMINAE

In *Ischaemum rugosum* each spike is many jointed and each joint has a sessile and a pedicelled spikelet. In *Apluda mutica* the inflorescence is a leafy panicle made up of many solitary, simple racemes or false spikes

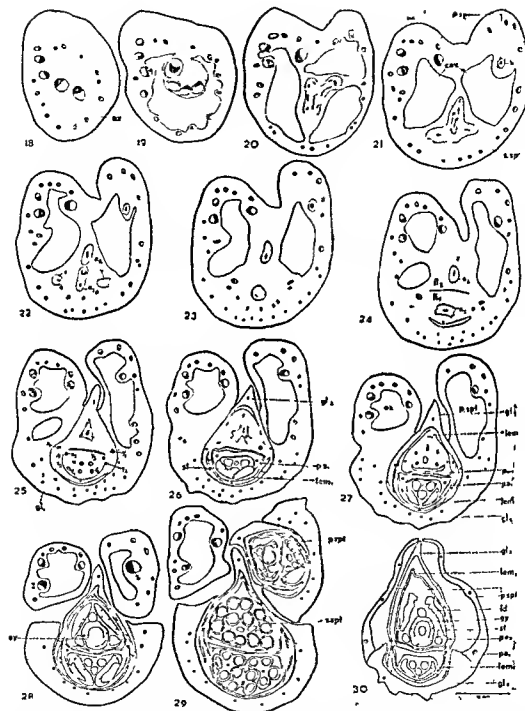
Spikelets are present in threes—one sessile and two pedicelled. Generally, one of the pedicelled spikelets is fertile while the other is imperfect and is represented by one or two glumes only. In both the grasses the sessile spikelet has a lower male floret and an upper bisexual perfect floret besides the two empty glumes. The pedicelled spikelet may be similar to the sessile one or may be reduced.

Both the grasses resemble very much in the vasculature of the spikelets. Here, only the vascular supply of *Ischaemum rugosum* is described. In a transverse section of the stalk region of the spike there are a number of small bundles on the peripheral side and four large bundles lying in a row in the centre (Fig. 18). At the base of a joint there is a cavity in the centre (Fig. 19). Two of the large bundles migrate to the centre and form a vascular plexus (Figs. 19 and 20). From this plexus one bundle diverges out on the side on which two large bundles are already present (Fig. 20). These three bundles along with other smaller ones reconstitute the vascular supply of the axis (Fig. 21). The central plexus of vascular tissue now gives out a stout trace 'b' on the other side and with the peripherals already present there, constitutes the vascular supply of the stalk of the pedicelled spikelet (Figs. 21 and 22).

The vascular tissue left in the centre after the departure of trace 'b' supplies the sessile spikelet (Fig. 21). It sends off a number of minute traces towards the peripheral side which along with those already present there go to supply the outermost coriaceous glume (Figs. 22–24). Now the vascular tissue gives off one bundle on either side (X and Y) and then splits up into two vascular masses 'a<sub>1</sub>' and 'a<sub>2</sub>', one for each flower (Fig. 22). The bundles 'X' and 'Y' split into three each (x<sub>1</sub>, x<sub>2</sub>, x<sub>3</sub>, y<sub>1</sub>, y<sub>2</sub> and y<sub>3</sub>) of these 'x<sub>2</sub>' and 'y<sub>2</sub>' form the lateral nerves of lemma I; 'x<sub>3</sub>' 'y<sub>3</sub>' form the two nerves of the beaked palea of lemma I while 'x<sub>1</sub>' and 'y<sub>1</sub>' again split into three each and supply lateral veins to the glume II, lemma II and its palea (Figs. 25–27).

The median nerves to lemmas I and II are provided by the two vascular masses 'a<sub>1</sub>' and 'a<sub>2</sub>' respectively (Fig. 24). After the departure of median nerve to lemma I, the vascular tissue 'a<sub>1</sub>' destined to supply the lower flower, sends out two traces l<sub>1</sub> and l<sub>2</sub> for the two lodicules and then supplies one trace to each of the three stamens (Figs. 25 and 26). A minute trace left in the centre supplies the rudimentary gynoeceum which is occasionally present (Fig. 26). The lodicular bundles as usual divide into several minute strands (Figs. 27 and 28).

The vascular tissue a<sub>2</sub> meant for the upper floret after supplying the lodicules and the stamens supplies the gynoeceum in the usual manner (Fig. 28).



FIGS. 18-30 Figs 18-29 *Ischaemum rugosum*—Serial transverse sections of the spike from base upwards. Fig. 30. *Apluda mutica*—TS of the spike passing through the sessile spikelet. (cav, cavity; fl, flower; s.spt, sessile spikelet.)

The stalk of the pedicelled spikelet has a large bundle 'b' derived from the central plexus of vascular tissue and this constitutes the main vascular supply of this spikelet (Fig. 28). The vascular supply of the sessile spikelet of *Apluda mutica* is just similar to that of *T. ruigosum*. However, there are two pedicelled spikelets at a joint (Fig. 30).

In the material studied here the pedicelled spikelet is exactly similar to the sessile one (Fig. 29). Arber (1934), however, described the pedicelled spikelet in this species as imperfect. Hooker (1897) also described it as quite variable.

#### Sub-tribe: ROTTBOELLINAE

A characteristic feature of the members of this sub-tribe is the concrecence of the pedicel portion of the pedicelled spikelet with the axis of the spike. In *Hemarthria compressa*, *Rottboellia exaltata*, and *Mnesithea laevis*, at each joint there is a sessile spikelet and a perfect or imperfect pedicelled spikelet. In *Ophiuros exaltatus* there is no trace of the pedicelled spikelet. In *Hemarthria compressa* the sessile spikelet has two outer empty glumes and two florets; the lower is reduced to lemma and the upper is perfect and bisexual.

In the stalk portion of the spike there is a peripheral ring of small bundles and in the centre there are four large bundles lying in two groups of two each (Fig. 31). Just below the nodal region the four central bundles come closer, anastomose and give out one stout trace 'a' for the sessile spikelet and one 'b' for the pedicelled spikelet (Figs. 32 and 33). The bundle 'b' migrates towards the periphery and along with the peripheral bundles already present there constitutes the vascular supply of the stalk of the pedicelled spikelet (Figs. 33-35). The bundle 'a' meant to supply the sessile spikelet expands to form a vascular tissue which soon sends off a number of traces to the outermost empty glume which is coriaceous (Figs. 34 and 35). The central vascular tissue now gives off two bundles ( $x$  and  $y$ ) one on either side which ultimately split into three each ( $x_1, x_2, x_3; y_1, y_2$  and  $y_3$ ; Figs. 35-37). Out of these ' $x_1$ ' and ' $y_1$ ' form the marginals of second glume. The median nerve for this glume is derived directly from the central vascular mass. The bundles ' $x_2$ ' and ' $y_2$ ' enter the base of lemma I and the remaining ones ' $x_3$ ' and ' $y_3$ ' supply the lemma II (Figs. 36 and 37). Both the lemmas are devoid of median nerves. The palea of the second floret is hyaline and non-vascular and remains united at the base with the lodicules by its margins (Fig. 37). The central vascular mass now supplies the rest of the organs in the usual manner. In a few gynoecea a very small outgrowth was noticed on the posterior side

at the junction of the stylar branches with ovary proper reminding the condition observed in *Triticum aestivum* (Chandra, 1962 a)

At the base of the pedicelled spikelet proper, the bundle 'b' (derived from the bundles of the main axis) migrates towards the centre and expands to form a mass of vascular tissue (Fig 37) which supplies the different organs as in the case of sessile spikelet

In *Rottboellia exaltata* each joint has a sessile and a pedicelled spikelet. The stalk of the pedicelled spikelet is adnate to the axis throughout its length. Each joint has a ball like structure at the base by means of which it fits into the concavity or socket of the joint below

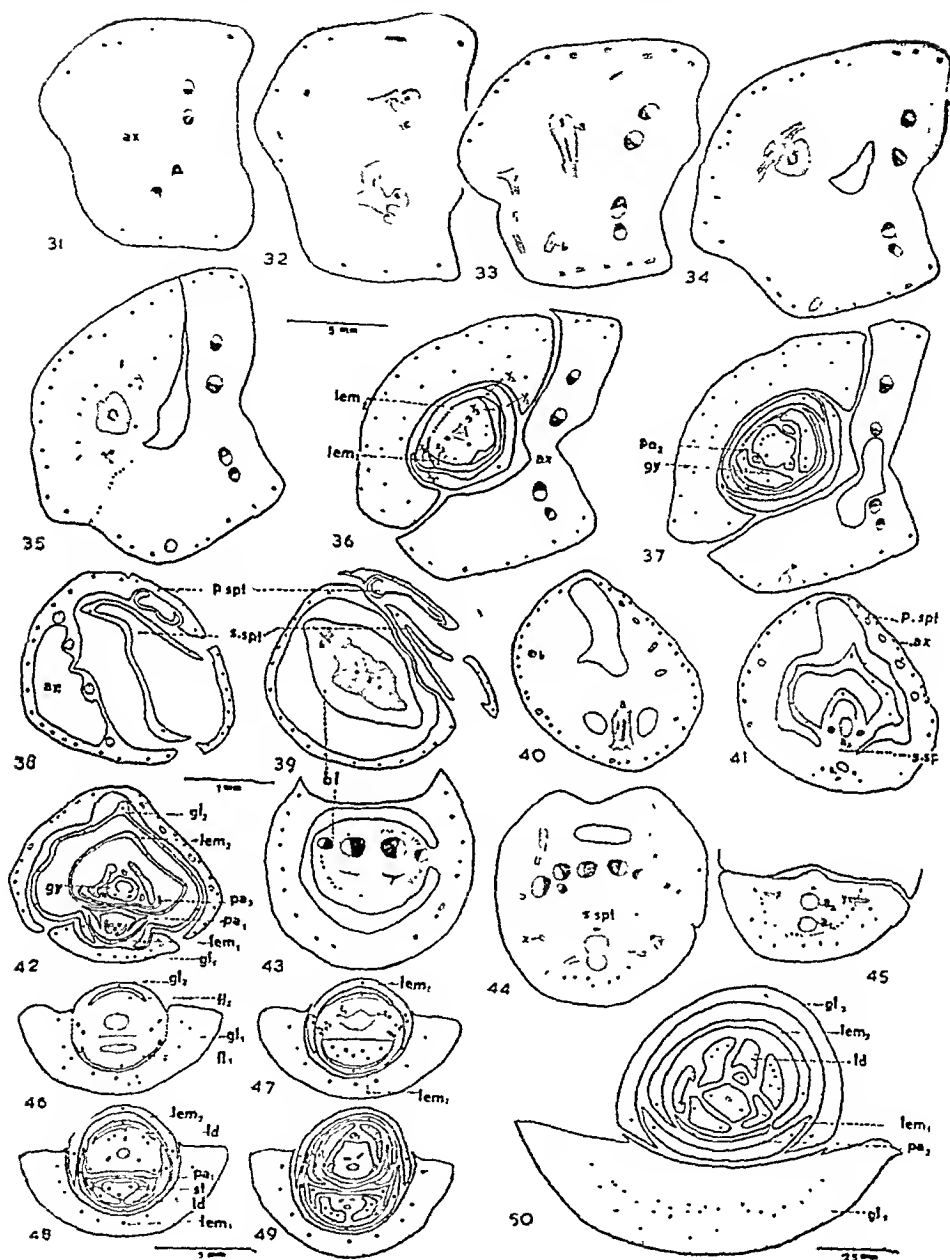
In the axis of the spike there are four large central bundles and a number of small peripheral bundles (Fig 38). This seems to be a constant feature of all the grasses of the sub-tribe Rottboellinae studied here. Just below the node, the four central bundles migrate into the ball like structure to form a plexus of vascular tissue (Fig 39). This plexus as in *Hemarthria comorensis* gives out a stout trace 'a' and another minute branch 'b' and then splits up into the four large bundles which continue into the axis (Figs 40 and 41). The stout strand 'a' along with some peripheral bundles constitutes the vascular supply of the sessile spikelet while the bundle 'b' along with 4 or 5 peripheral bundles constitutes the vascular supply of the pedicelled spikelet.

The sessile spikelet has two florets, a lower staminate and an upper hermaphrodite. The strand 'a' expands to form a mass of vascular tissue which after supplying the outer two empty glumes splits into two groups ( $a_1$  and  $a_2$ ) meant respectively for the lower and the upper floret (Fig 41). The vascular supply of the individual floret resembles that in other grasses (Fig 42).

The pedicelled spikelet is generally imperfect. The bundle 'b' as usual forms a mass of vascular tissue at the base of spikelet proper and supplies the first, second glumes and other vestigial organs.

In *Ophiuros exaltatus* the spike has a number of joints each having a ball like structure at the base and a concavity at the top. As in *Rottboellia exaltata* and *Mnesithea leavis* this ball fits into the cavity of the joint below. The solitary sessile spikelet present at each node has two florets, an imperfect lower and a perfect hermaphrodite upper one.

In a transverse section of the spike just below a joint there are a number of small bundles towards the peripheral side of the axis and four or five large bundles towards the inner side (Fig. 43). At the base of a joint the four or



FIGS. 31-50. Figs. 31-37. *Hemarthria compressa*—Serial transverse sections of the spike from base upwards. Figs. 38-42. *Rottboellia exaltata*—Transsections of the spike from base upwards. Figs. 43-49. *Ophiuros exaltatus*. Figs. 43 and 44. Transsections of the spike. Figs. 45-49. Transsections of the sessile spikelet from base upwards. Fig. 50. *Mnesithea laevis*—Transsection of the sessile spikelet showing six lodicules. (bl, ball-like structure.)



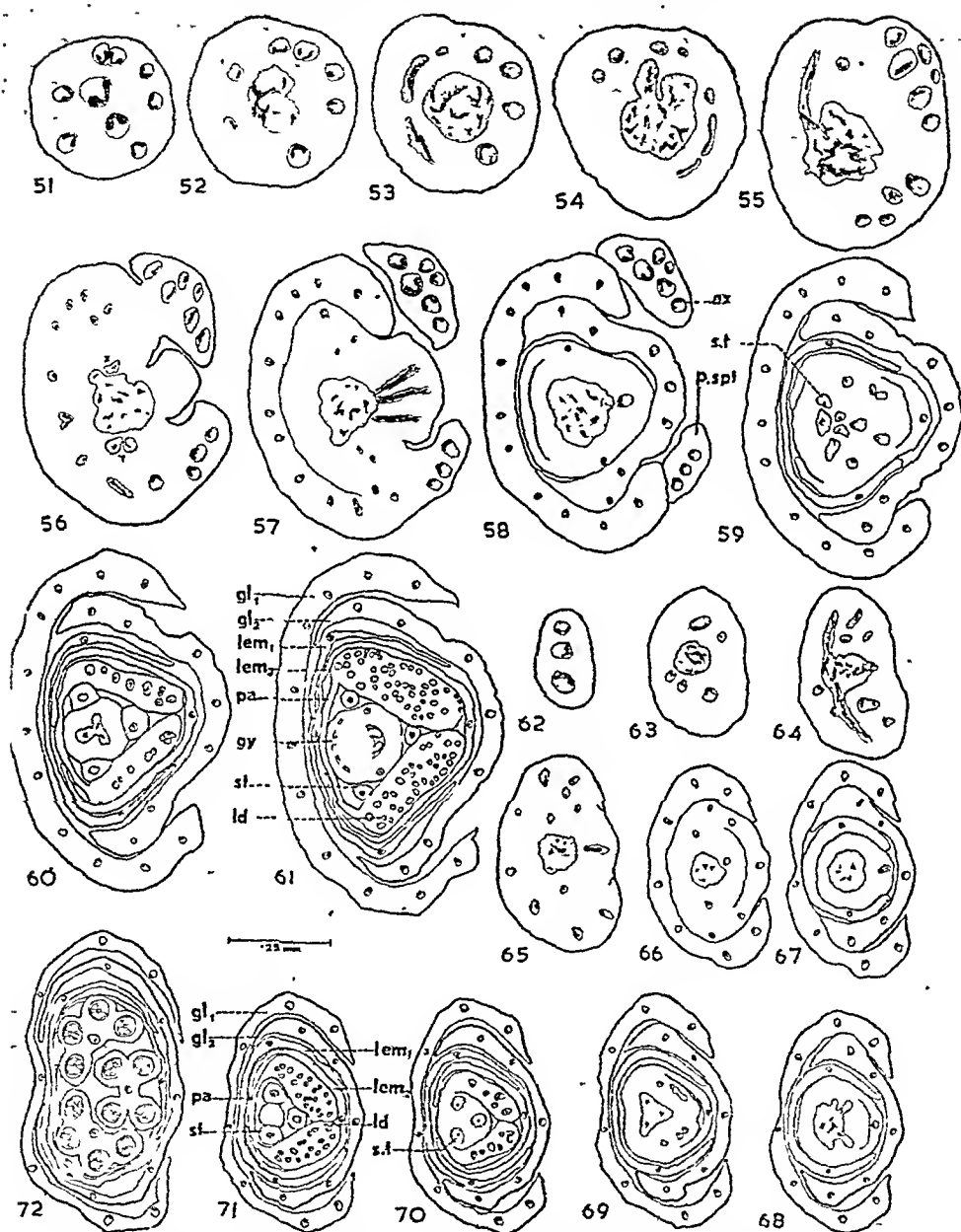
five large bundles come in the centre and enter the ball of the upper joint where they form a plexus of vascular tissue. At the nodal region a prominent trace diverges out from the central plexus into the cortex for the solitary sessile spikelet. Soon after divergence it gives off a number of traces to the outermost empty glume which is very thick and coriaceous (Fig 44). The vascular tissue left behind gives out three traces—one median on the side opposite the first glume and two laterals ( $x$  and  $y$ ). The remaining mass of vascular tissue now resolves into two components ' $a_1$ ' and ' $a_2$ ' for the lower and upper floret respectively (Fig 45). The bundles ' $x_1$ ' and ' $y_1$ ' resolve into four each which go as lateral nerves to second empty glume lemma I, lemma II and palea of lemma II (Figs 46-49). Median traces to lemma I and II are provided by the vascular masses ' $a_1$ ' and ' $a_2$ ' respectively which supply other organs of the floret in the usual manner.

A detailed study of the morphology and vascular anatomy of the spike and spikelets of *Mnesithea laevis* has already been made earlier (Chandra, 1958). Since then in one spikelet six lodicules instead of the normal two have been observed (Fig 50). Out of these, the two antero laterals and one posterior were quite well developed and have rich vascular supply while the other three alternating to these were smaller and with lesser number of vascular bundles. The occasional appearance of six lodicules is an important feature as it has never been recorded earlier in any grass except for certain Bambuseae where it is found as a normal feature (see Rowlee, 1898).

#### Sub tribe SORGHINAE

The inflorescence in the Sorghinae is a panicle of racemes often few jointed. At each joint there is a pair of spikelets, a sessile and a pedicelled. The sessile spikelet is awned and bisexual while the pedicelled is awnless and male. Each spikelet is two flowered of which the lower is reduced to its lemma.

In *Sorghum halepense* there are two large bundles in the centre in the rachis and a number of smaller bundles on the outer side (Fig 51). At the base of a joint the two central bundles coalesce and supply the sessile spikelet (Figs 52-55). The outer bundles divide and three on one side enter the pedicel of the pedicelled spikelet while the rest continue in the rachis (Fig 55). The central vascular tissue supplying the sessile spikelet gives out a number of traces to the outer empty glume which is very thick and coriaceous (Figs 56 and 57). The stele again gives out one trace on either side ( $x$  and  $y$ , Fig 56), and then supplies three traces on the anterior side for the second empty glume (Fig. 57). The two laterals soon split up into two each (Fig 57). One of



FIGS. 51-72. *Sorghum halepense*. FIGS. 51-58. Serial transverse sections of the spike from base upwards. FIGS. 59-61. Serial transverse sections of the sessile spikelet from base upwards. FIGS. 62-72. Serial transverse sections of the pedicelled spikelet from base upwards.

these on either side along with one trace derived earlier enter the second empty glume which is thus 7-nerved (Figs. 57 and 58). The remaining

two bundles one on either side enter the lemma I. The central mass of vascular tissue again gives out a median trace for the lemma II which is fertile and awned (Figs. 58 and 59). The remaining mass of vascular tissue gives one trace to each of the two lodicules and subsequently three traces to the three stamens and then supplies the gynoeceium, in the usual manner (Figs. 59-61). The two lateral bundles continue into the stigmas. The palea of lemma II is non-vascular and hyaline. The lodicular traces as usual divide and subdivide to form a large number of minute bundles. The vascular supply of the pedicelled spikelet is essentially the same as that of the sessile spikelet (Figs. 62-72). The glume II is, however, 5-nerved and the gynoeceium of the upper floret is represented by a pistillode (Fig. 71). The lateral bundles of lemma I and glume II arise conjointly.

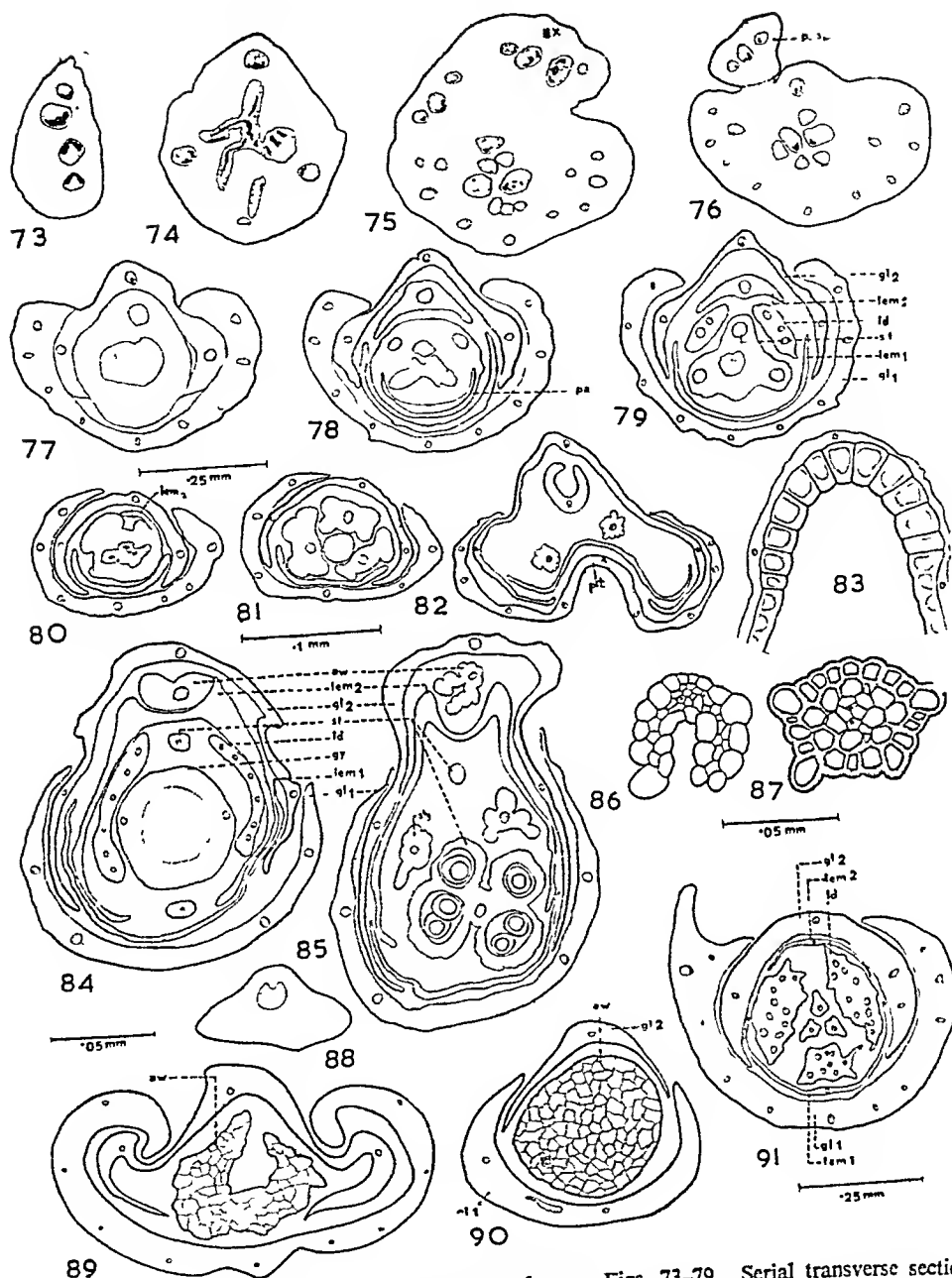
*Vetiveria zizanioides* resembles *Sorghum halepense* in the vascular anatomy of the spikelets.

#### Sub-tribe: ANDROPOGONINAE

In all the members of the sub-tribe Andropogoninae studied here the rachis is many-jointed except for *Themeda* and *Iseilema* in which there are only a few joints. At each joint there is a sessile and a pedicelled spikelet. The sessile spikelet is awned and hermaphrodite while the pedicelled spikelet is awnless and staminate.

In *Dichanthium annulatum* there are present four bundles in the rachis which anastomose and divide (Figs. 73 and 74). Three on one side migrate for the pedicelled spikelet while four bundles continue into the rachis (Figs. 75 and 76). The mass of vascular tissue which is destined to supply the sessile spikelet soon gives out a number of bundles for the outer empty glume and three bundles, one median and two laterals for the second empty glume (Fig. 77). The central mass of vascular tissue again gives out a trace for the lemma II which is continued into an awn (Fig. 78). The lemma I and the palea of the lemma II are hyaline non-vascular structures (Fig. 78). The vascular supply to the two lodicules, stamens and gynoeceium is as usual (Fig. 79). In the case of pedicelled spikelet the lemma II is also non-vascular and hyaline (Figs. 80-81). In *Bothriochloa pertusa* there is a pit present on the outer empty glume (Figs. 82 and 83).

In *Arthraxon ciliaris* there are only two stamens which are antero-posterior and are of unequal height. Another important feature is that the awn is a lateral off-shoot of lemma II (Figs. 84 and 85). It may be recalled that in most of the members of the Tribe Andropogoneae it is a terminal structure,



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Figs. 73-91. Figs. 73-81. *Dichanthium annulatum*. Figs. 73-79. Serial transverse section of the rachis and sessile spikelet from base upwards. Figs. 80-81. Transsections of the pedicelled spikelet. Figs. 82-83. *Bothiochloa pertusa*. Fig. 82. T.S. of the sessile spikelet from the upper portion. Fig. 83. Portion of the glume I enlarged showing the pit. Figs. 84-87. *Arthraxon ciliaris*. Figs. 84-85. Transsections of the sessile spikelet. Figs. 86-87. Transsections of the awn. Figs. 88-91. *Heteropogon contortus*. Figs. 88. T.S. awn. Figs. 89-90. Transsections of the apical portion of the sessile spikelet showing the awn. Fig. 91. T.S. pedicelled spikelet showing three lodicules. (aw, awn; stig, stigma.)

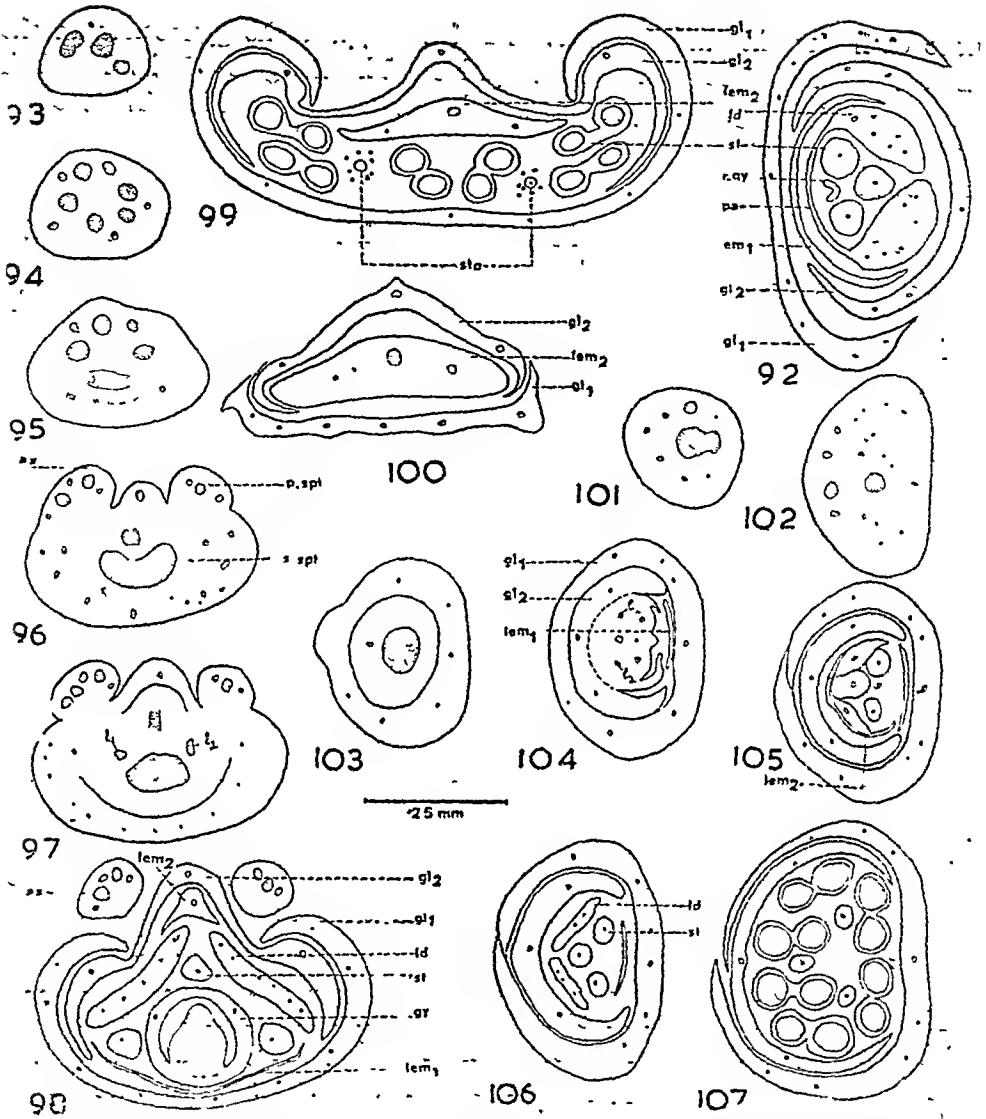
being a direct continuation of the second lemma. In a cross-section, the awn at a lower level is horse-shoe-shaped while at a slightly higher level it has thick-walled cells on the outer side enclosing a few thin-walled cells (Figs. 86 and 87).

In *Heteropogon contortus* the sessile and the pedicelled spikelets in the lower region of the spike are all identical and staminate. Each spikelet has two glumes, two lemmas, the lower sterile and the upper subtending a male flower. In the remaining portion of the rachis there are heterogamous pairs, a sessile female spikelet and a pedicelled male one. The general plan of the vascular supply resembles that of *Dichanthium annulatum*. In one of the male spikelets there were three lodicules instead of the normal two. Such a condition has also been recorded earlier in *Mnesithea laevis* (Chandra, 1958). The lemma II is produced into an awn in the apical region. In apical region of the spikelet in a transverse section the lemma has a mid-rib region with a stout vascular bundle and with margins curved towards the gynoecium. Higher up these margins are curved in opposite direction. The vascular bundle also becomes horse-shoe-shaped in cross-section. In this transitional region of the lemma there are only thin-walled cells which are bigger in size and are wavy in outline. In the apical region, the awn has a central vascular strand embedded in thick-walled tissue (Figs. 88-90).

*Cynlopogon martinii* resembles *Dichanthium annulatum* in all essential details. The pedicelled spikelet (Fig. 92), however, has got three glumes ( $gl_1$ ,  $gl_2$  and *lem. I*), a rudimentary palea (*pa*) which is fused with the two lodicules at the base by its margins, three stamens and a vestigial gynoecium. An important feature is the absence of lemma II while its subtended flower is present.

In *Themeda* sp. the panicle has clusters of racemes subtended by a boat-shaped spathe. Each raceme is subtended by a leaf-like structure, the spatheole and is composed of two joints the lower having a sessile hermaphrodite and a pedicelled staminate spikelet and the upper with a sessile bisexual and two pedicelled male spikelets. The lemma II of both the sessile spikelets is produced into an awn.

There are a number of bundles left after the departure of vascular supply to the pedicelled spikelet and the rachis (Figs. 93-96). These bundles supply the sessile spikelet. Some of them unite to form a central mass of vascular tissue while the others rearrange themselves on the periphery and pass out into the outermost glume which is thick and coriaceous with inflexed margins (Figs. 96 and 97). The second glume has three bundles. A nerveless and



FIGS. 92-107. Fig. 92. *Cymbopogon martinii*.—T.S. of the pedicelled spikelet showing the absence of lemma II. Fig. 93-107. *Themeda* sp. Figs. 93-100. Serial transverse sections of the rachis and the sessile spikelet from base upwards. Figs. 101-107. Serial transverse sections of the pedicelled spikelet from base upwards.

hyaline lemma I is present opposite the second glume. The central vascular tissue now sends off a stout trace on the anterior side for lemma II which is reduced to its midrib region only (Figs. 97 and 98). Two traces now diverge out from the central vascular mass in the antero-lateral direction for the two lodicules (Fig. 97). The remaining mass of vascular tissue gives out one

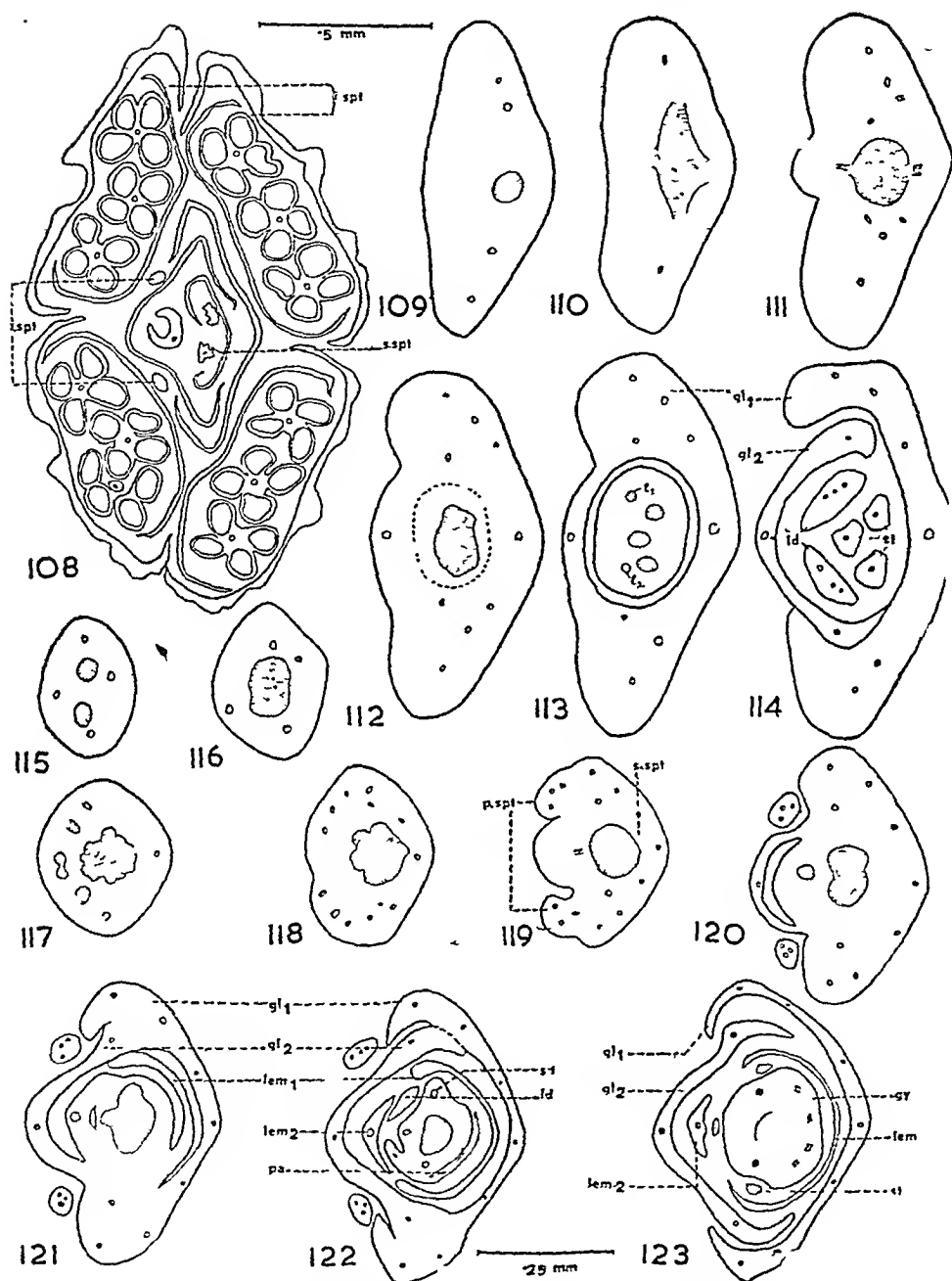
trace each for the three stamens and then supplies the gynoecium in the usual manner. Palea is absent altogether. The margins of the I and II glumes are very tightly appressed together (Figs 98 and 99). The median nerve of lemma II divides into many branches in the awn region (Figs 99 and 100).

In a pedicelled spikelet in the stalk region there are a number of bundles which multiply at the base of the spikelet proper (Figs 101 and 102). Three or four bundles fuse on one side to form a mass of vascular tissue while the others migrate into the outermost glume which is thick and remains united at the base by its margins (Figs 103). The vascular tissue gives out one median and two laterals for the second glume (Fig 104). Lemma I and II are present but are vestigial structures (Fig 105). The palea of lemma II is also absent. The remaining vascular mass supplies the two lodicules and stamens in the usual manner (Figs 106 and 107).

In *Iseilema anthephoroides* the panicle consists of racemes occurring in clusters. Each raceme has an involucrel whorl of 4 male spikelets which are shortly stalked. The involucrel spikelets enclose a central awned female spikelet which has got a pair of pedicelled spikelets at the base each having a long pedicel surmounted by a male spikelet (Fig 108).

Each involucrel spikelet has two glumes, two lodicules and three stamens. There is no trace of the lemmas and paleas of the two flowers. In the pedicel portion there is one large central bundle and one or two smaller bundles on either side of it (Fig 109). All the bundles except for the marginal one on either side coalesce to form a mass of vascular tissue (Fig 110). This vascular tissue gives out a number of minute traces towards one side which along with the two already present there go to supply the outermost glume (Figs 111-114). The second glume gets one median and two lateral nerves one on either side. These two glumes remain united at the base (Fig 113). The central mass of vascular tissue after supplying the two glumes gives out two traces ( $l_1$  and  $l_2$ ) on the antero-lateral side for the two lodicules and then splits up into three traces for the three stamens (Figs 113 and 114). The lodicular bundles as usual divide into three to five minute branches.

After supplying the involucrel spikelets the axis has two large bundles and four smaller ones (Figs 115). The two large bundles unite to form a vascular tissue (Figs 116 and 117). The three small bundles also divide and out of the resulting bundles three on either side migrate into the pedicels of the pedicelled spikelets. Rest of the small bundles enter the fused basal portion of the 1st and 2nd glumes of the central female spikelet (Figs 118-121).



FIGS. 108–123. *Iseilema anthephoroides*. Fig. 108. T.S. of the spike showing 4 involucra spikelets, one sessile spikelet and two pedicels of the pedicelled spikelets. Figs. 109–114. Serial transverse sections of the involucral spikelet from base upwards. Figs. 115–123. Serial transverse sections of the rachis and the sessile spikelet from base upwards. (*i.spt*, involucral spikelet.)



The 2nd glume gets one median bundle from the central vascular tissue also. After the detachment of the two outer glumes, the lemma I separates off as a hyaline non-vascular structure. A single strong median trace now diverges out from the central mass of vascular tissue for the lemma II which is produced into an awn (Figs 120-123). Two rudimentary lodicules and a palea may be present but they are non vascular (Fig 122). The vascular tissue left in the centre supply the gynoecium in the usual manner. The stamens are, however, vestigial.

The pedicelled spikelets when present resemble the involucrel spikelets but in the racemes sectioned here for study they were reduced to their pedicel portions only.

### DISCUSSION

*Vascular Anatomy of the Spikelet*—It will be worthwhile to describe here briefly the general ground plan of vascular supply of the spikelet in the Andropogoneae (Fig 124). Let us consider first of all those spikelets in which the lower floret is reduced to its lemma as in the majority of taxa belonging to this tribe. In this case the vascular tissue meant to supply a spikelet after giving out a number of traces for the outermost empty glume gives out one bundle on either side ( $x$  and  $y$ ) and then furnishes mid-veins to the glumes and lemmas. The bundles ' $x$ ' and ' $y$ ' divide and supply lateral nerves to the glumes, lemmas and paleae. The remaining vascular mass supplies two traces to the two lodicules, three traces to the three stamens and then supplies the gynoecium in the usual manner.

In those cases where the lower floret is also developed and is staminate as in the subtribe Ischiminae and some of the Rottboellinae, the vascular tissue after giving out the lateral bundles ' $x$ ' and ' $y$ ' splits into two vascular masses ' $a_1$ ' and ' $a_2$ ' meant respectively for the lower and upper floret. In *Ischaemum rugosum* and *Apluda mutica* the lateral bundles of the glumes, the lemmas and the two paleae are derived from the bundles ' $x$ ' and ' $y$ '.

It follows that in the majority of the Andropogoneae where the lower floret is reduced to its lemma, the bundles of the second palea arise conjointly with the lateral bundle of the two lemmas and the second empty glume. In some of the members, however, the palea and lower lemma are non vascular and hyaline. In those members where the lower floret is also present the bundles of both the paleae, the lateral bundles of the two lemmas and in some cases those of the glumes arise in the form of a single vascular mass on either side ( $x$  and  $y$ ).

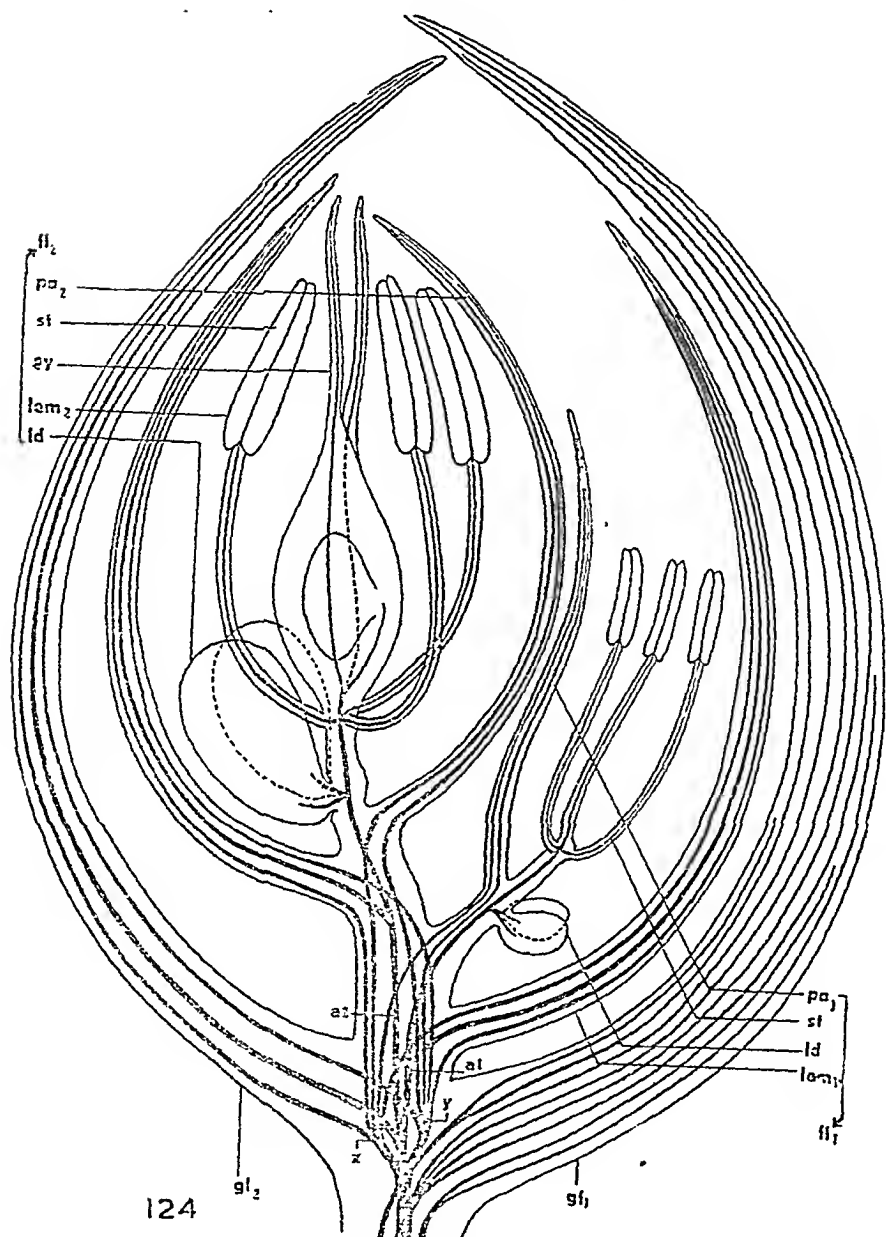


FIG. 124. Schematic representation of the vascular supply of the spikelet in the tribe Andropogoneae.

The vascular supply thus indicates that there has been an abbreviation of the axes in the spikelet. In the related tribe Paniceae also it has been concluded that there has been an abbreviation or telescoping of the rachilla

as well as the floral axes (Chandra, 1962 *a*), so much so, that the bundles of the plaça (which belong to the floral axis) arise along with the laterals of the lemma

*Trends of Specialization in the Spike and Spikelets*—As will be recalled there are two types of spikelets in this tribe—the sessile and the pedicelled ones. At each node of the spike there is a sessile and a pedicelled spikelet except for the terminal portions of spikes where in some cases, there may be two pedicelled spikelets accompanying a sessile one. In *Apluda* we find two pedicelled and a sessile spikelet as constituting the spike. In *Mnesithea laevis* there are two sessile and a reduced pedicelled spikelet at each joint (Chandra 1958). The pedicel of the pedicelled spikelet is either free or is conerescent with the axis of the joint as in the sub tribe Rottboellinae. In some of the highly specialized grasses e.g., *Iseilema* there is another set of involueral spikelets which resemble the pedicelled ones on being male or reduced. Arber (1934) correlated the pedicelled character of the spikelet with sterility. As an example, she cites *Ischaemum rugosum* where the pedicelled spikelet is reduced to various extents in different parts of the spike. In some species studied here *Apluda mutica*, *Hemarthria compressa* and the members of the Saccharinae the pedicelled spikelet is developed to the same extent as its sessile companion. In the members of the Sorghinae and the Andropogoninae the pedicelled spikelet has generally the lower floret reduced to its lemma and the upper being staminate. In *Rottboellia exaltata* the pedicelled spikelet is further reduced and is represented by a few glumes only. In *Mnesithea laevis* it is represented by the conerescent pedicel portion, occasionally having some rudimentary organs (Chandra, 1958). In *Ophiuros exaltatus* there is no trace whatsoever of the pedicelled spikelet and there is only one sessile spikelet at each joint.

The spikelet in the Andropogoneae is basically two flowered with the upper floret, generally perfect and bisexual in sessile spikelet and staminate in the pedicelled. The lower floret is either staminate or reduced to its lemma. There is a marked tendency towards the suppression of the lower floret in the whole of the sub family Panicoideae. In some cases as in *Heteropogon contortus*, *Iseilema antheporoides*, etc., some of the sessile spikelets of a spike may be female with vestigial stamens only while pedicelled spikelets are generally staminate. Thus we find that there is an urge towards a segregation of sex in the Andropogoneae. The pedicelled spikelets tend to be male in the majority of cases while the sessile one is either female or male (*Heteropogon contortus*). It seems that this tendency for separation of sexes finds

its ultimate expression in the tribe Maydeae where there are separate male and female spikelets which, as in maize, are grouped in separate inflorescences.

There is yet another marked tendency in this tribe towards the elaboration of the outer empty glumes which are very thick and sometimes coriaceous. On the other hand the barren lemma of the lower floret and the palea of the upper floret tend to be hyaline and nonvascular as in the majority of grasses studied here. In some cases the palea is completely missing or is not very apparent due to its vestigial nature. The second lemma of the sessile spikelet is generally produced into an awn but as pointed out earlier the awn is a lateral structure in *Arthraxon ciliaris* and is not a direct continuation of the lemma II. The pedicellate spikelets which are generally male are awnless. In *Cymbopogon martinii* the lemma of the upper floret of the pedicelled spikelet is conspicuously absent although its subtended flower is present. In *Imperata cylindrica* the spikelet shows further reduction as both the lemmas and the palea of lemma II are nonvascular, lodicules are absent and there are only two stamens placed antero-laterally. In *Arthraxon ciliaris*, however, the two stamens are antero-posterior and are of unequal height, a unique feature in the Gramineae. The ultimate reduction of the spikelet is reached in the case of the involucre spikelets as those of *Iseilima anthephoroides* where there are only two firm outer glumes, two lodicules and three stamens in a spikelet, the entire lower floret along with the lemma and palea of the upper floret being altogether wanting.

During the course of the present investigation certain abnormalities have been recorded. Increased number of lodicules has been recorded for *Mnesithea laevis* and *Heteropogon contortus*. In *H. contortus* there were three lodicules in one instance instead of the normal two. The third lodicule occupied a posterior position recalling the condition obtained in the bamboos. Such a condition has earlier been recorded for *Mnesithea laevis* (Chandra, 1958) and *Sorghum halepense* (Long, 1930). One abnormal spikelet of *M. laevis* was found to have six lodicules in a flower. Three of these, two antero-lateral and one posterior, were better developed and better vascularized than the other three alternating with them. The occasional presence of six and three lodicules as has been observed here, lends some support to the inference that the lodicules represent perianth members of the inner whorl and that the outer whorl and the posterior member of the inner whorl have become suppressed during the course of evolution.

In some spikelets of *M. laevis* it has been observed that there is an occasional increase in the number of glumes (Chandra, 1958). Instead of two

outer glumes and two lemmas there were in one case two outer empty glumes and three lemmas out of which the uppermost was fertile. It has also been found that in a few spikelets of this grass the rachilla is produced beyond the upper fertile floret as a minute non vascular outgrowth. Randolph (1936) also found, in the pistillate spikelets of *Zea mays*, a rudimentary rachilla extending beyond the insertion of the fertile floret in between the paleae of the fertile and sterile flowers. Such examples seem to indicate that the two-flowered spikelet of the Panicoideae in general has been derived through reduction from a multiflowered spikelet. Further reduction of the lower floret after the attainment of two flowered condition has resulted in the uni flowered spikelet.

### SUMMARY

The present paper deals with the vascular anatomy of the spike and spikelets of twenty species of Andropogoneae. The vascular anatomy of the spikelet indicates that there has been an abbreviation of the axes in the spikelet. In the majority of the Andropogoneae where the lower floret is reduced to its lemma, the bundles, of the second palea arise conjointly with the lateral bundle of the two lemmas and the second empty glume. In those members where the lower floret is also present the bundles of both the paleae, the lateral bundles of the two lemmas and in some cases those of the glumes arise in the form of a single vascular mass on either side.

Attention has been drawn to the various trends of specialization in the spike and spikelets of this tribe. There is a marked tendency for the imperfection of the pedicelled spikelet which may be variously reduced and in some cases is entirely unrepresented. There is yet another tendency towards the elaboration of the outer empty glumes and the suppression of the lemma I and the palea II which tend to be hyaline and non vascular and in some cases are completely wanting.

Occasional increase in the number of lodicules has been recorded for *Mnesithea laevis* and *Heteropogon contortus*.

### ACKNOWLEDGEMENT

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# THE PRODUCTION OF VITAMIN B<sub>12</sub>-LIKE SUBSTANCES BY MARINE MICRO-ORGANISMS

## I General Survey

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## INTRODUCTION

MARINE BACTERIA are known to bring about various chemical changes in the sea, they mineralise organic matter (Wakeman, Carey and Reuszer, 1933), oxidise ammonia to nitrate, transform sulphur compounds, influence the phosphorous cycle (Renn, 1937), and are important geological agents which influence the diagenesis of sedimentary materials in many ways

According to ZoBell and Feltham (1938), the bacteria also serve as an important source of food for certain animals and according to Luck, Sheets and Thomas (1931), even protozoa. Since the availability of the nutrients in the sea may be insufficient for the growth of marine life, the bacteria probably provide the necessary nutrients through the products of their metabolic activity

In addition, accessory growth factors or vitamins may be synthesized by bacteria (Burkholder, 1959). Vitamin B<sub>12</sub> activity has been reported in sea water by several workers like Daisely and Fisher (1938), Starr (1956), Kashiwada, Kakimoto, Kanazawa and Kawazoe (1957) and Daisely (1957). Since the vitamin is synthesized by micro organisms only, its presence in the sea is probably of microbial origin.

Accordingly, an attempt was made to isolate and identify some marine organisms and survey their ability to synthesize vitamin B<sub>12</sub>.

## METHODS

Sea water samples were collected from various places between 18° 00' N-72° 00' E and 18° 52' N-72° 85' E in previously sterilized glass stoppered bottles, following the method adopted by numerous early workers like Johnstone (1892), Heydenreich (1899), Abbott (1921), Whipple (1927) and Zillig (1929).

The sea is inhabited by many micro-organisms, differing widely in their cultural requirements; hence more than one medium was utilized for their isolation. The four different media utilized for the isolation of bacteria were:

1. Sea-water agar.
2. Nutrient agar with sea-water base.
3. Nutrient agar with 3% NaCl.
4. ZoBell's medium 2216 (1946).

To isolate yeasts from sea-water a special method, similar to the one used by Kachwalla (1953), was adopted. This enrichment culture technique was done with Sucrose Yeast Extract medium. An attempt was also made to isolate actinomycetes using Czapek's agar as modified by Dox (1910) and Conn's agar (1916) media.

All the isolated cultures were assayed for their vitamin B<sub>12</sub> activity, using the test organism *Lactobacillus leichmannii* 313 (7830), the method adopted being the one recommended by "The United States Pharmacopoeia, 14th supplement, 1950". *Lactobacillus leichmannii* 313 (7830) was used as the test organism, since *Euglena gracilis* var. *bacillaris*, the organism specific for cyanocobalamin failed to grow in the presence of the sample. This may be attributed to the presence of some inhibitory factor produced by the marine micro-organisms.

Vitamin B<sub>12</sub> was liberated in the broth by adding 1 mg./ml. of sodium metabisulphite to the broth culture and steaming for 15 minutes, as recommended by Pinto (1955).

A tentative identification of the active isolates was then attempted, on the basis of their morphological and cultural characteristics on various media according to standard methods (Breed, Murray and Smith, 1957 and ZoBell and Upham, 1944).

## RESULTS

The isolation procedures resulted in the isolation of 131 pure cultures from 35 sea-water samples. These included 5 cultures of yeasts and 5 of Actinomycetes.

After assaying, 103 isolates were found to possess vitamin B<sub>12</sub>-like activity varying from 0.1–5.80 mμg./ml. However, the yeasts and actinomycetes failed to exhibit any vitamin-B<sub>12</sub> like activity.



Among the active isolates 57% were Gram positive in nature and 43% were Gram negative. They fell into four groups

- 1 Gram positive spore bearing bacilli
- 2 Gram positive cocci
- 3 Gram positive non spore bearing bacilli
- 4 Gram negative bacilli

Tables I-IV give the different groups with the strains identified up to species wherever possible, and the vitamin B<sub>12</sub> like activity in the culture filtrate

TABLE I  
*Identity and vitamin B<sub>12</sub> like activity of Gram-positive  
spore bearing bacilli*

Isolate No	Identity	Vitamin B <sub>12</sub> -like activity in µg/ml
A <sub>1</sub>	<i>Bacillus circulans</i>	0.26
C <sub>1</sub> , I <sub>1</sub> , Q <sub>1</sub>	<i>Bacillus megaterium</i>	0.15-0.31
D <sub>1</sub> , F <sub>1</sub> , L <sub>1</sub> , Z <sub>1</sub> , BB <sub>1</sub>	<i>Bacillus cereus</i>	0.15-0.76
E <sub>1</sub> , N <sub>1</sub>	<i>Bacillus borborykoides</i>	0.15-0.78
E <sub>2</sub>	<i>Bacillus carotiflagellatus</i>	0.17
E <sub>3</sub> , I <sub>2</sub>	<i>Bacillus submarinus</i>	0.26-5.0
E <sub>4</sub> , O <sub>1</sub> , N <sub>2</sub>	<i>Bacillus pumilus</i>	0.18-0.26
E <sub>10</sub> , L <sub>2</sub> , M <sub>1</sub> , M <sub>2</sub> , N <sub>3</sub> , Q <sub>2</sub> , Y <sub>1</sub> , AA <sub>1</sub>	Genus <i>Bacillus</i>	0.19-1.10
K <sub>1</sub>	<i>Bacillus coagulans</i>	0.32
L <sub>4</sub> , M <sub>3</sub>	<i>Bacillus abyssus</i>	0.17-0.18
N <sub>4</sub> , O <sub>2</sub> , P <sub>1</sub>	<i>Bacillus unguis</i>	0.17-1.20
P <sub>2</sub> , R <sub>1</sub>	<i>Bacillus thalassokoides</i>	0.15-1.00
R <sub>1</sub> , T <sub>1</sub> , U <sub>1</sub> , Z <sub>2</sub>	<i>Bacillus epiphytus</i>	0.19-1.10
V <sub>1</sub>	<i>Bacillus filicollis</i>	0.18
AA <sub>1</sub>	<i>Bacillus subtilis</i>	0.30
BB <sub>1</sub>	<i>Bacillus leicheniformis</i>	0.29

TABLE II

*Identity and vitamin B<sub>12</sub>-like activity of Gram-positive cocci*

Isolate No.	Identity	Vitamin B <sub>12</sub> -like activity in mμg./ml.
A <sub>2</sub> , A <sub>3</sub>	<i>Micrococcus colpogenes</i>	0.44-0.52
D <sub>3</sub>	<i>Micrococcus caseolyticus</i>	0.24
E <sub>2</sub> , J <sub>1</sub> , R <sub>4</sub> , Y <sub>1</sub>	Genus <i>Micrococcus</i>	0.15-2.15
F <sub>5</sub>	<i>Micrococcus luteus</i>	0.29
Q <sub>3</sub>	<i>Micrococcus sedentereus</i>	0.60
Q <sub>4</sub>	Genus <i>Sarcina</i>	0.35
V <sub>3</sub>	<i>Micrococcus sedementeus</i>	1.65
Z <sub>1</sub>	<i>Micrococcus conglomeratus</i>	0.17

TABLE III

*Identity and vitamin B<sub>12</sub>-like activity of Gram-positive non-spore-bearing bacilli*

Isolate No.	Identity	Vitamin B <sub>12</sub> -like activity in mμg./ml.
B <sub>4</sub> , E <sub>9</sub> , H <sub>2</sub> , U <sub>2</sub>	Genus <i>Brevibacterium</i>	0.18-1.90
G <sub>2</sub>	<i>Brevibacterium sociovivum</i>	2.0
H <sub>4</sub>	<i>Brevibacterium minutiferula</i>	0.55

## DISCUSSION

Vitamin B<sub>12</sub> is the only vitamin which is synthesized almost exclusively by micro-organisms. Among those showing high synthesizing activity are the actinomycetes and members of the Bacillaceae family. *Bacillus megatherium* is an excellent vitamin B<sub>12</sub> producer yielding 0.8 μg./ml. (Garibaldi,

Ijichi, Snell and Lewis, 1953). Leviton and Hargrove (1952) reported *Propionibacterium freudenreichii* yielding 3-4 µg./ml. Burton and Lochhead (1951) reported a yield of 1 µg./ml. from *Rhizobium meliloti*. Species of *Mycobacterium*, *Flavobacterium*, *Lactobacillus*, *Corynebacterium* and *Clostridium* are some of the other micro-organisms that exhibit this activity. However, during the present investigations, species of Actinomycetes isolated from sea-water did not exhibit any activity. The isolates, showing comparatively higher activity, belonged to the genera *Pseudomonas*, *Achromobacter*, *Micrococcus* and *Brevibacterium*.

TABLE IV

*Identity and vitamin B<sub>12</sub>-like activity of Gram-negative bacilli*

Isolate No	Identity	Vitamin B <sub>12</sub> -like activity in µg./ml.
A <sub>1</sub>	<i>Pseudomonas calcis</i>	0.20
B <sub>1</sub> , J <sub>1</sub>	<i>Pseudomonas felthami</i>	0.56-1.0
B <sub>2</sub>	<i>Pseudomonas ambigua</i>	0.40
B <sub>3</sub> , M <sub>2</sub>	<i>Pseudomonas ichthyodermis</i>	0.36-0.88
C <sub>2</sub> , C <sub>3</sub> , F <sub>1</sub> , F <sub>2</sub> , F <sub>4</sub> , I <sub>1</sub> , K <sub>1</sub> , M <sub>4</sub> , N <sub>1</sub> , O <sub>2</sub> , P <sub>2</sub> , Q <sub>2</sub> , R <sub>1</sub> , S <sub>1</sub> , BB <sub>2</sub>	Genus <i>Pseudomonas</i>	0.19-5.80
D <sub>1</sub> , D <sub>2</sub>	<i>Pseudomonas azlogena</i>	1.15-5.0
E <sub>11</sub>	<i>Pseudomonas obscura</i>	0.28
G <sub>2</sub>	<i>Pseudomonas streotrophi</i>	2.62
H <sub>1</sub>	<i>Pseudomonas membranula</i>	5.80
T <sub>2</sub>	<i>Pseudomonas pleomorpha</i>	0.25
C <sub>2</sub>	<i>Achromobacter pestifer</i>	3.20
D <sub>2</sub> , K <sub>2</sub>	<i>Achromobacter thalassius</i>	0.17-0.30
I <sub>2</sub> , K <sub>2</sub> , BB <sub>2</sub>	Genus <i>Achromobacter</i>	0.50-5.50
Y <sub>2</sub>	<i>Achromobacter stationis</i>	0.31
D <sub>4</sub>	Genus <i>Flavobacterium</i>	0.54
H <sub>2</sub>	<i>Flavobacterium fucatum</i>	0.85
K <sub>4</sub>	<i>Flavobacterium marinoviriosum</i>	0.28
B <sub>2</sub> , V <sub>1</sub>	Genus <i>Vibrio</i>	0.20-1.70
X <sub>2</sub>	Genus <i>Photobacterium</i>	0.15-0.36
N <sub>2</sub>	<i>Spirillum virginianum</i>	0.80

Since *L. leichmannii* is known to give response to deoxyribosides and in particular thymidine besides vitamin B<sub>12</sub>, a differential assay with alkali-treated samples was carried out, which showed that all the *L. leichmannii* activity in the sample was due to vitamin B<sub>12</sub>-like substances and the possibility of any substances other than cobalamins was thus eliminated.

Marine bacteria are reported to be highly versatile physiologically and are believed to be capable of attacking nearly any kind of organic substrate and many inorganic compounds (ZoBell, 1946). In the course of the present study it was found that a majority (77.6%) of the isolates were able to ferment glucose with the formation of acid while only two of them produced gas. According to ZoBell and Grant (1943) all heterotrophic marine bacteria are able to assimilate glucose although only 46 out of 60 cultures tested by ZoBell and Upham (1944) formed acid from glucose and none of them produced gas. In contrast with the soil and freshwater bacteria, only 11.6% of the isolates fermented lactose. Other sugars fermented by the isolates were sucrose (67%), maltose (57.2%), mannitol (45.6%), glycerol (29.1%), arabinose (20.4%) and xylose (12.5%).

Marine bacteria are also reported to be able to attack most kinds of proteinaceous materials (ZoBell, 1946). Nearly all the isolates were found to be able to liberate ammonia from peptone and about 63% could liquefy gelatine. However, only a few (10%) were able to liberate detectable quantities of indole from tryptophan. Although many workers like Bavendamm (1932), Bertel (1935), ZoBell and Feltham (1935) and ZoBell (1946) have reported abundance of urea fermenters in the sea, only about 6% of the isolates were found to decompose urea with the formation of ammonia. Most of the pioneer workers like Russell (1893), Fischer (1894), Vernön (1898), Gazert (1906) and Gräf (1909) have demonstrated in the sea bacteria capable of reducing nitrates. ZoBell and Upham (1944) found 34 of their 60 species with the same ability. In our experiments 63 of the 103 isolates were able to reduce nitrate to nitrite. Casein, starch and fats were also found to be hydrolysed by many of the isolates.

Microbial transformation of sulphur compounds is also one of the chief functions of the bacteria inhabiting marine environments. About 50% of the isolates were found to be capable of forming hydrogen sulphide. Ellis (1932) demonstrated from 10,000 to 3,000,000 saprophytes per gram of bottom deposits from the Clyde Sea, nine-tenths of which liberated hydrogen sulphide from albuminous materials. ZoBell (1938) found 10,000 to 1,000,000 hydrogen sulphide producers per gram of bottom sediments from the Pacific Ocean off the coast of California.

The data collected so far have shown that the sea does contain a bacterial flora capable of synthesizing vitamin B<sub>12</sub> like factors and these bacteria must be the sources supplying the vitamin needs of various forms of life present in the marine environment

### SUMMARY

One hundred and thirty-one cultures were isolated from 35 sea-water samples and assayed for vitamin B<sub>12</sub>-like activity using *Lactobacillus leichmannii* 313 (7830). The active isolates were identified up to the species wherever possible adopting standard methods

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# THE PRODUCTION OF VITAMIN B<sub>12</sub>-LIKE SUBSTANCES BY MARINE MICRO-ORGANISMS

## II. Studies on Nutrition

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## INTRODUCTION

THE bacterial flora of the sea is characterised by exceptional biochemical versatility. Marine micro-organisms are reported to be able to produce extra-cellular organic substances (Lucas, 1955; Lewin, 1956 and Fogg, 1937) as well as certain intracellular substances like vitamin B<sub>12</sub> (Starr, Jones and Martinez, 1957).

Such metabolic activities of these micro-organisms are however greatly influenced by the environmental conditions. The presence of a favourable substrate is one of the most important factors influencing the metabolism of the organisms. It could, therefore, be possible to alter one or the other biological properties by varying the substrate composition.

The production of antibiotics has been reported to be affected by the nutrition of the streptomycetes (Johnstone and Waksman, 1947; Duleney, 1948; Woodruff and Ruger, 1948 and Perlman and Obrien, 1956). Studies have also been carried out on the vitamin B<sub>12</sub> synthesis and the nutritional requirements of these organisms (Garibaldi *et al.*, 1951; Wood and Hendlin, 1952 and Kurz and Nielsen, 1958).

A variety of carbon and nitrogen sources have been used by different research workers for vitamin B<sub>12</sub> production. Various carbohydrates include glucose (Petty and Matrishin, 1950; Burton and Lochhead, 1951), fructose (Garibaldi *et al.*, 1951), maltose (Petty and Matrishin, 1950), xylose (Wood and Hendlin, 1952), sucrose (Garibaldi *et al.*, 1951; Halbrook, Cords, Winter and Sutton, 1950). Alcohols like glycerol (Garibaldi *et al.*, 1951), sorbitol, mannitol (Petty and Matrishin, 1950) and other complex carbohydrates like soluble starch, cane and beet molasses (Garibaldi *et al.*, 1951 and Wood and



Hendlin, 1952), and malt extract (Petty and Matrishin, 1950) have also been used

Many complex nitrogenous materials have also been reported for vitamin B<sub>12</sub> synthesis. Wood and Hendlin (1952) have reported the use of beef extract, blood meal, distiller's solubles, dried yeast, fish meal, fish solubles, casein and its enzyme digests, salmon waste meal and yeast extract. The use of soya bean meal was reported by Burton and Lochhead (1951) and many others (Hodge, Hansen and Allgeier, 1952, Saunders, Otto and Sylvester, 1952, Squibb and Sons, 1953, Hester and Ward, 1954; Pagano and Greenspan, 1954 and Pfeifer, Vognovich and Heger, 1954). Other substances used are defatted mustard, oilseed cakes (Chatterjee, *et al*, 1957), copra meal (Hodge, Hansen and Allgeier, 1952), peptone (Petty and Matrishin, 1950 and Burton and Lochhead, 1951), skimmed milk and whey (Review of Chemical and Engineering News, 1949) and dried grain slop (Bennett, 1954).

Various workers have also reported the usefulness of growth factors in the production of antibiotics, enzymes and vitamin B<sub>12</sub>. Gunsalus and Bellemy (1944) observed that pyridoxal stimulated the formation of certain enzyme systems in bacteria. Costa-Pereira (1962) found thiamine useful for enhancing the production of antibiotics from actinomycetes. Hall and Tsuchiya (1950) during their study on *Flavobacterium decolorans* found that the addition of PABA, niacin and calcium pantothenate to the medium enhanced the production of the vitamin factor.

Hence, the study of the effect of different substrates and mineral salts on the growth and vitamin B<sub>12</sub> synthesis was undertaken with 10 isolates on the basis of their vitamin B<sub>12</sub> synthesizing ability. A detailed study was also made of the effect of various carbon and nitrogen sources on the growth and the production of vitamin B<sub>12</sub>-like factors with one selected isolate.

#### MATERIALS AND METHODS

The ten different isolates selected for the study were

- B<sub>1</sub> belonging to genus *Brevibacterium*
- C<sub>1</sub> *Achromobacter pestifer*
- D<sub>1</sub> *Pseudomonas azotogena*
- G<sub>1</sub> *Pseudomonas stercorotropha*
- H<sub>1</sub> *Pseudomonas membranula*
- I<sub>1</sub> belonging to genus *Pseudomonas*
- I<sub>2</sub> belonging to genus *Achromobacter*

N<sub>4</sub> belonging to genus *Pseudomonas*.

V<sub>3</sub> *Micrococcus sedementus*.

Y<sub>1</sub> belonging to genus *Micrococcus*.

Their vitamin B<sub>12</sub>-like activity ranged from 1.90–5.80 µg./ml. in broth filtrate.

The eight different media used were :—

- (a) Czapek's medium (modified by Dox, 1910).
- (b) Conn's medium (Conn, 1916).
- (c) Farben-fabriken Bayer's medium (Farben-fabriken Bayer, 1952).
- (d) Burton and Lochhead's medium (Kerrawala, 1958).
- (e) Synthetic sea-water medium (ZoBell, 1946).
- (f) Hall's medium (Hall and Tsuchiya, 1951).
- (g) Saunder's medium (Saunders, Otto and Sylvester, 1952).
- (h) Coconut cake medium (Pinto, 1955).

For the mineral salt studies, the synthetic medium of Farben-fabriken Bayer was selected. The effect of nine different salts was studied by omitting each salt at a time and observing its effect on the growth and the vitamin synthesis.

The carbohydrates selected for the study were arabinose, rhamnose, glucose, fructose, mannose, galactose, maltose, lactose, soluble starch, dextrin, glycerol, mannitol, dulcitol, sorbitol and inositol. The complex sources of carbon included malt extract, molasses, corn syrup and jaggery.

The basa medium consisted of essential mineral salts and casein hydrolysate. The carbon source in the original medium totalling 1.25% was substituted by the test carbohydrate in the same concentration. The complex sources were incorporated in 1% concentration.

The various nitrogen sources included inorganic nitrogen sources like NaNO<sub>3</sub> and NH<sub>4</sub>Cl, organic amides like asparagine and urea and various amino-acids. The complex nitrogen sources of vegetable origin were gram meal, soya bean meal, millet meal, sesamum oilcake, coconut cake, white pea, mung (*Phaseolus radiatus*), wheat meal, corn steep liquor and wheat bran. Those of animal and microbial origin were peptone, tryptone, bacto-peptone, proteolysed liver, casein hydrolysate, meat extract, yeast extract and skimmed milk.

The usefulness of riboflavin, choline, folic acid, inositol, calcium pantothenate, nicotinic acid and *para*-amino benzoic acid was also investigated.

The nitrogen sources were incorporated in the medium on the basis of 70 mg of  $N_2$  per 100 ml of the basal medium wherever possible. The complex sources of vegetable origin were used in the form of aqueous extract of the flour. A 10% solution of the meal was autoclaved at 15 lb pressure for half an hour. The resulting paste was filtered through lint. The filtrate obtained was made up to its original volume. This aqueous extract was used instead of water in the preparation of media. The substances of animal origin were added in 1% concentration. The vitamins were added to the synthetic medium in concentrations of 20  $\mu$ g/ml.

### RESULTS AND DISCUSSION

During the study on growth and production of vitamin  $B_{12}$ -like activity in various media, excellent results were obtained on the synthetic medium of Farter fabriken Bayer as seen in Table I. The growth as well as the production of vitamin  $B_{12}$  like activity in Czepek's and Conn's media was very poor. Since Burton and Lockhead's and synthetic sea-water media were highly nutritive, both the growth and production of the vitamin activity were good, though the response of all the isolates was not uniform. With the complex media it was observed that, to some extent, Hall's and Saunder's media were well utilized for the production of vitamin  $B_{12}$ -like factors, though, in general, the growth was not satisfactory.

From the results obtained on Farter fabriken Bayer's medium and the synthetic sea water medium it was seen that the presence of various mineral salts had a very significant effect on the growth as well as the vitamin activity.

Table II indicates the effect of various mineral salts, and from this it can be safely concluded that the presence of various mineral constituents is very essential to the growth as well as the synthesis of the vitamin  $B_{12}$ -like factors and the absence of one or the other of the constituent minerals considerably affects the growth and/or the vitamin  $B_{12}$  synthesis.

This may be attributed to the presence of various mineral salts and various excretory products of marine life in the sea water. Nutrition and metabolism of marine bacteria have been studied in detail by Macleod, Onofrey and Norris (1954), Macleod and Onofrey (1956-57) and Tomlinson and Macleod (1957) and certain metallic ions have been known to enhance the growth of marine micro-organisms. Thus Farter fabriken Bayer's and synthetic sea water media could serve as a rich source of mineral salts and the variation was reflected in the growth as well as the production of the vitamin  $B_{12}$ -like factors.

TABLE I

Vitamin B<sub>12</sub>-like activity of the ten isolates in various media. Vitamin B<sub>12</sub>-like activity in  $\mu\text{g./ml.}$  in broth filtrate as assayed by L. leichmanni and Growth in media Nos.

Isolate No.	1	2	3	4	5	6	7	8
	Growth Vit.	Growth Vit.	Growth Vit.	Growth Vit.	Growth Vit.	Growth Vit.	Growth Vit.	Growth Vit.
B <sub>4</sub>	+	—	+++	++	+++	++	+	+++
C <sub>4</sub>	++	0.25	+++	++	+++	+++	++	+++
D <sub>1</sub>	++	0.30	+++	+++	+++	+++	+++	+++
G <sub>3</sub>	++	—	+++	+++	+++	+	++	++
H <sub>3</sub>	++	—	+++	+++	+++	+++	++	+++
I <sub>2</sub>	++	0.30	+++	+++	+++	+++	++	+++
I <sub>3</sub>	++	0.55	+++	+++	+++	+++	+++	+++
N <sub>4</sub>	+	—	+++	+++	+++	+++	+++	+++
V <sub>3</sub>	+	—	+++	+++	+++	+	++	+++
Y <sub>1</sub>	++	0.21	+++	+++	+++	+++	++	+++

+ = Poor.

++ = Fair.

+++ = Good.

++++ = Very good.

1. Czapek's Medium.

2. Conn's Medium.

3. Farben-fabriken Bayer's Medium.

4. Burton and Lochhead's Medium.

5. Synthetic Sea-water Medium.

6. Hall's Medium.

7. Saunders's Medium.

8. Coconut cake Medium.

TABLE II

*The effect of mineral salts on vitamin B<sub>12</sub>-like activity and growth of the ten isolates Vitamin B<sub>12</sub>-like activity in µg/ml. in broth filtrate with the omission of*

Identity	NaCl	(NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	K <sub>2</sub> HPO <sub>4</sub>	MgSO <sub>4</sub> 7H <sub>2</sub> O	CaCl <sub>2</sub>
	Growth Vit	Growth Vit	Growth Vit.	Growth Vit.	Growth Vit.
B <sub>4</sub>	+ -	+ -	++ 0.75	+ 0.25	++ 1.00
C <sub>4</sub>	+ -	+ -	+++ 0.95	++ 0.55	+++ 2.00
D <sub>1</sub>	++ 1.75	+++ 1.10	+++ 1.00	++ 1.25	+++ 2.80
G <sub>2</sub>	+ 0.75	++ -	++ 0.80	++ 1.10	++ 1.40
H <sub>2</sub>	+ 1.50	++ 2.10	+++ 1.00	++ 1.00	++ 1.25
I <sub>1</sub>	+ 1.10	++ 2.60	+++ 1.60	+ 1.25	+++ 2.90
I <sub>2</sub>	+ 1.10	++ 2.10	+++ 1.00	++ 1.00	+++ 2.35
N <sub>4</sub>	+ 0.50	++ 0.65	++ 0.80	+ 0.25	++ 0.75
V <sub>2</sub>	+ -	+ -	++ -	++ 0.50	++ 0.50
Y <sub>1</sub>	++ 0.60	++ 1.25	+++ 0.95	++ 0.35	+++ 1.00
Identity	FeSO <sub>4</sub> 7H <sub>2</sub> O	ZnSO <sub>4</sub> 7H <sub>2</sub> O	CaCl <sub>2</sub> 6H <sub>2</sub> O	MnSO <sub>4</sub> 4H <sub>2</sub> O	Control
	Growth Vit.	Growth Vit.	Growth Vit.	Growth Vit.	Growth Vit.
B <sub>4</sub>	+ -	++ -	++ -	+++ -	+++ 1.80
C <sub>4</sub>	+ 0.15	++ 0.65	+++ 0.15	+++ 0.60	+++ 3.00
D <sub>1</sub>	++ 0.85	+++ 2.00	+++ 0.10	+++ 1.10	+++ 5.40
G <sub>2</sub>	+ -	++ -	++ -	+++ -	+++ 2.50
H <sub>2</sub>	++ 0.90	+++ 2.10	+++ 0.10	+++ 2.00	+++ 5.60
I <sub>1</sub>	++ 0.85	+++ 2.00	++++ 0.25	++++ 2.55	++++ 5.80
I <sub>2</sub>	++ 1.25	+++ 2.35	++++ 0.20	++++ 2.35	++++ 5.70
N <sub>4</sub>	+ -	+ -	++ -	+++ 0.60	+++ 1.50
V <sub>2</sub>	+ -	+ -	++ -	+++ 0.75	+++ 1.60
Y <sub>2</sub>	+ 0.25	+++ 0.90	++ -	+++ -	++++ 2.25

The control values were obtained with the inclusion of all the salts in the medium.  
The salts were used at their initial concentrations.

+ = Poor. +++ = Good.  
++ = Fair ++++ = Very good.

TABLE III

*The effect of carbohydrates on the growth and  
vitamin B<sub>12</sub>-like activity of I<sub>3</sub>*

Carbohydrate		Growth	Vitamin in m $\mu$ g./ml.
1.	Arabinose .. ..	++	2.00
2.	Rhamnose .. ..	++++	5.10
3.	Glucose .. ..	+++	3.10
4.	Fructose .. ..	+++	3.50
5.	Mannose .. ..	++	2.25
6.	Galactose .. ..	++++	5.35
7.	Maltose .. ..	++++	5.00
8.	Lactose .. ..	+	1.90
9.	Soluble starch ..	++	1.25
10.	Dextrin .. ..	++	3.85
11.	Glycerol .. ..	++	2.10
12.	Mannitol .. ..	++	2.65
13.	Dulcitol .. ..	++	2.00
14.	Sorbitol .. ..	++++	5.35
15.	Inositol .. ..	+++	3.85
16.	No carbohydrate ..	+	..

The complete failure of the isolates to produce the factors in the absence of cobalt is probably due to its being an essential precursor for the synthesis of cobalamins.

The observations in Table III show the weakly saccharolytic nature of the selected isolate. The growth varied considerably with individual carbohydrates, affecting the production of vitamin B<sub>12</sub>-like factors also. Rhamnose, galactose, maltose and sorbitol were utilized better than the others, whereas lactose and soluble starch served as very poor carbon sources. Although various carbohydrates have been recommended for vitamin B<sub>12</sub> production

only few have been effective mostly in the case of *Streptomyces* and *Escherichia* strains.

Among the complex carbon sources tried (Table IV) malt extract gave the best results, but the values obtained were much lower than those obtained with the carbohydrates.

TABLE IV  
*The effect of complex carbon sources on growth  
and vitamin B<sub>12</sub>-like activity of I<sub>2</sub>*

Carbon source	Growth	Vitamin in µg/ml in broth filtrate
1 Jaggery	+++	3.50
2. Molasses	+++	3.50
3 Corn syrup	++++	3.75
4 Malt extract	+++	4.00

The results obtained with various kinds of synthetic nitrogenous sources are presented in Table V. This study revealed the inability of the inorganic salts to enhance the production of vitamin B<sub>12</sub>-like factors, although the growth was good. However, the organic amides, urea and asparagine gave slightly better results. Among the amino acids, histidine and proline gave the best results, while lysine and tryptophan had detrimental effects on the production of the vitamin factor but the growth was not affected. The three sulphur-containing amino acids tested gave very poor results, especially methionine, in the presence of which the isolate completely failed to produce the vitamin factors suggesting a vitamin opposing action of the organism, a similar observation having been reported by Ayres (1960). In general, it can be seen that the results agree with the previous observations made by Ostreiff and Henry (1939), regarding the utilization of simple amino-acids as sole sources of nitrogen.

The results obtained with the vegetable and animal nitrogenous sources can be observed in Table VI. In the former case the best values were obtained with gram meal, soya bean meal, sesamum oil-cake, white Pea and mung

TABLE V

*Effect of various synthetic  $N_2$ -compounds on growth and vitamin  $B_{12}$ -like activity of  $I_3$*

Nature of compound and identity		Growth	Vitamin in $\mu\text{g./ml.}$ in broth filtrate
1. Inorganic $N_2$ Source			
(a) $\text{NaNO}_3$	.. ..	+++	3.35
(b) $\text{NH}_4\text{Cl}$	.. ..	+++	3.00
2. Organic Amides			
(a) Asparagin	.. ..	+++	3.85
(b) Urea	.. ..	+++	4.75
3. Amino-acids			
(a) Histidine	.. ..	+++	5.00
(b) Arginine	.. ..	+++	4.00
(c) Lysine	.. ..	+++	2.00
(d) Proline	.. ..	+++	4.70
(e) Serine	.. ..	+++	3.90
(f) Tryptophan	.. ..	+++	2.10
(g) Threonine	.. ..	+	1.10
(h) Leucine	.. ..	++++	5.10
(i) Isoleucine	.. ..	++++	5.50
(j) Alanine	.. ..	++++	4.75
(k) Glycine	.. ..	++++	4.00
(l) Valine	.. ..	+++	4.60
(m) Aspartic acid	.. ..	+++	5.25
(n) Glutamic acid	.. ..	++++	4.75
(o) Tyrosine	.. ..	+++	5.00
(p) Phenylalanine	.. ..	+++	4.50
(q) Methionine	.. ..	+++	..
(r) Cystine	.. ..	+++	2.10
(s) Cysteine	.. ..	+++	2.00
(t) Nil	.. ..	+	..



TABLE VI

*The effect of vegetable and animal N<sub>2</sub> sources on growth and vitamin B<sub>12</sub>-like activity of I<sub>3</sub>*

N <sub>2</sub> Source				Growth	Vitamin in µg./ml. in broth filtrate
<b>A. Vegetable Source</b>					
1.	Gram meal	..	..	++++	5.10
2.	Soyabean meal	..	..	++++	5.45
3.	Millet meal	..	..	+++	3.00
4.	Sesamum oilcake	..	..	++++	5.65
5.	Coconut cake	..	..	+++	3.90
6.	White pea	..	..	++++	5.00
7.	Mung ( <i>Phaseolus radiatus</i> )	..	..	++++	5.90
8.	Wheat meal	..	..	+++	4.00
9.	Corn steep liquor	..	..	+++	2.25
10.	Wheat bran	..	..	+++	4.30
11.	Nil	..	..	+	..
<b>B. Animal Source</b>					
1.	Peptone	..	..	++++	5.70
2.	Tryptone	..	..	++++	5.65
3.	Bacto peptone	..	..	++++	5.55
4.	Proteolysed liver	..	..	++++	6.15
5.	Casein hydrolysate	..	..	++++	5.60
6.	Meat extract	..	..	++++	5.40
7.	Skimmed milk	..	..	+	..
8.	Nil	..	..	+	..
<b>C. Microbial Source</b>					
1.	Yeast extract	..	..	++++	6.20

(*Phaseolus radiatus*). Coconut cake, wheat meal and corn steep liquor gave very low values, which was quite contrary to Kerrawala's (1958) observations.

that these gave excellent results during her study on vitamin B<sub>12</sub> production by actinomycetes. Among the various animal and microbial nitrogen sources, yeast extract and proteolysed liver gave the best values. The results obtained with the latter can be attributed to the fact that liver itself is a good source of the factor as well as its precursors. On the other hand, skimmed milk hindered the production of the vitamin factor, which could be expected, since the organism had failed to proliferate in it during the identification experiments. The overall effect of both the nitrogenous sources was good, the animal nitrogenous sources proving slightly more beneficial than those of vegetable origin.

The vitamins due to their growth-enhancing properties were found to exert a beneficial effect on the growth and the production of the vitamin B<sub>12</sub>-like factors (Table VII), calcium pantothenate and *para*-amino benzoic acid were found to have the highest beneficial effect. The rest of the vitamins gave lower values and to some extent had a slightly detrimental effect on the production of the vitamin factors. Hallbrook *et al.* (1950) also reported similar effects of choline on vitamin B<sub>12</sub> synthesis.

TABLE VII

*The effect of added vitamins on the growth and vitamin B<sub>12</sub>-like activity of I<sub>2</sub>*

Vitamin			Growth	Vitamin in μg./ml. in broth filtrate
1.	Riboflavin	..	+++	4.90
2.	Choline	..	++++	5.25
3.	Folic acid	..	+++	5.25
4.	Inositol	..	++	4.25
5.	Calcium pantothenate		+++	5.90
6.	Nicotinic acid	..	+++	5.45
7.	PABA	..	++++	6.25
8.	Nil	..	++++	5.72

Thus, a medium, rich in mineral salts, growth factors and complex nitrogen sources of animal origin and a suitable carbon source, would serve as an ideal substrate for the growth of marine bacteria and their production of vitamin B<sub>12</sub>-like factors

### SUMMARY

The effects of different substrates and the presence and absence of various mineral salts were studied with respect to the growth of and the synthesis of vitamin B<sub>12</sub>-like factors of ten different marine isolates. A detailed study was also made of the effect of various carbon and nitrogen sources on the growth and production of vitamin B<sub>12</sub> like factors by one selected isolate

### ACKNOWLEDGEMENTS

We are very much indebted to Dr D V Rege, Ph D (tech), Professor of Food Technology and Mr B M Mehta, B Sc (tech) of University Department of Chemical Technology, Bombay, for their helpful suggestions in the preparation of this paper

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# NEW RECORDS OF FUNGI FROM MYSORE

## III. Two Species of *Chaetospermum*

BY T. R. NAG RAJ

(Assistant Mycologist, Central Coffee Research Institute, Balehonnur, India)

Received October 3, 1963

(Communicated by Dr. L. Narayana Rao, F.A.Sc.)

### INTRODUCTION

THE genus *Chaetospermum* was established by Saccardo (1892) for a fungus at first described as *Tubercularia chaetospora* (Patouillard, 1888). Altogether, four species had been grouped under this genus since its inception, and recently, a new species has been reported from India under the name *C. camelliae* (Agnihothrudu, 1962). Except the last-named, no other species of the genus has been recorded from this country. Since August, 1960, the author has observed and made four gatherings of fungi belonging to the genus. This paper gives an account of these collections as new to this country and includes the author's comments on *C. camelliae*. Specimens have been deposited in the herbarium at Commonwealth Mycological Institute, England.

1. *Chaetospermum chaetosporum* (Pat.) Smith and Ramsb. in *Trans. Brit. Myc. Soc.*, 1913, 4, 318-30.

On rotting stems of *Dahlia* sp., Coffee Research Station, Balehonnur, India, 16-8-1962, T. R. Nag Raj, Herb. IMI 95833, Fig. 8.

The conidia in this collection measure  $30-42 \times 8-13 \mu$  with filiform setae, 7-12 in number at each pole, 32-65  $\mu$  long.

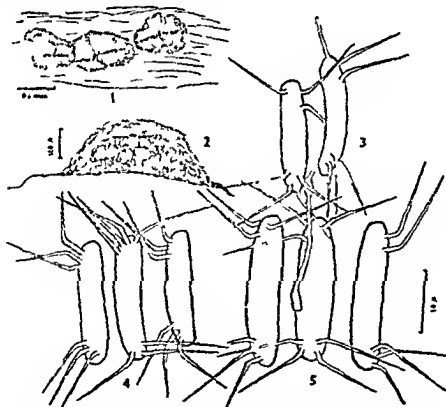
2. *Chaetospermum elasticae* Koord. In *Bot. Untersuch.*, Java, 1907, p. 244; *Syll. Fung.*, 10, 706.

The species was described from a collection made on decaying leaves of *Ficus elastica* in Kedu Province, Java.

The following description is based on the author's gatherings which are referable to this species:

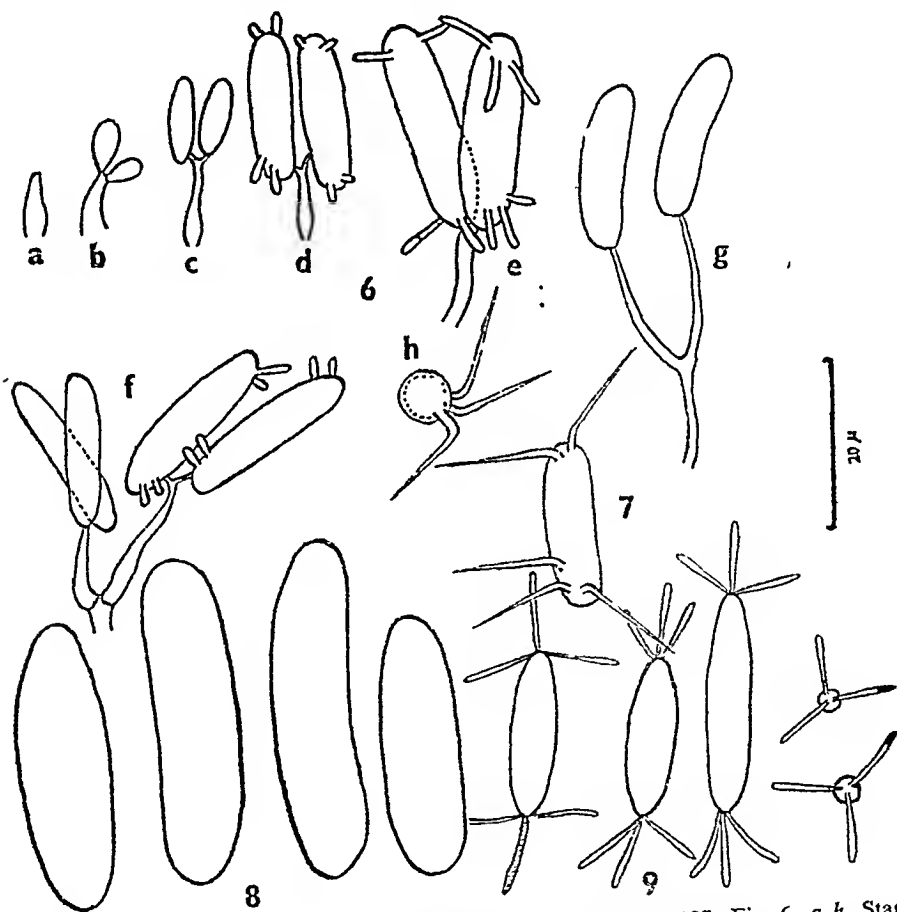
Pycnidia innate-crumpent, mostly scattered but occasionally gregarious and confluent, circular to irregular, hemispherical in section,  $300-350 \times 450-500 \mu$  in size, gelatinous, pearl white when moist, but yellowish-brown when dry, wall  $25-50 \mu$  thick, composed of interwoven, hyaline, branching septate hyphae, conidiophores arising from fertile hyphae lining the cavity of the pycnidium, filiform erect, hyaline, continuous, often attaining considerable lengths, conidia acrogenous, in clusters of two or occasionally three borne one slightly above the other, cylindrical with rounded or obtuse ends, straight or slightly curved, continuous, hyaline,  $20-25 \times 4-6 \mu$ , bearing 2-4 setae at each end, setae trichiform, hyaline, erect or slightly bent, developing on the lateral wall of the conidium, subapical,  $15-20 \mu$  long

On dead twigs of *Coffea canephora*, Coffee Research Station, 12-8-1960, T. R. Nag Raj Herb IMI 86739, Figs 1-5, on decaying bark of an unknown tree Coffee Research Station, 21-8-1960, T. R. Nag Raj, Herb IMI 99087, Figs 6-7



FIGS 1-5 *Chaetosporium elasticum* Herb IMI 86739 Fig. 1 Pycnidia, Fig. 2 Section of pycnidium, Fig. 3 Conidiophore with conidia, Fig. 4 Mature conidia from herbarium material, Fig. 5 Mature conidia from culture.

In the first collection the fungus was observed when the twigs were incubated in the laboratory in moist chambers for 72 hours at room temperatures; and a pure culture was obtained. In 7-8 days pycnidia appeared, at first as scattered minute bodies, later becoming gregarious and confluent, hemispherical to globose, erumpent and glistening white, acquiring a final dimension of 800-900  $\mu$  in diam. In culture, the conidia and setae have larger dimensions than those produced in nature. At maturity the pycnidia appear cupulate due to the apical wall becoming sunken or entirely evanescent. In the



FIGS. 6-9. Figs. 6 and 7. *Chaetospermum elasticum*. Herb. IMI 99087. Fig. 6, a-h. Stages of development of conidiophore, conidia and setae. a, conidiophore initial; b and c, development of young conidia on growing conidiophore; d and e, development of setae on conidia; f, two conidiophores bearing conidia in different stages of growth; g, elongation of the sterigmata (setae not shown); h, polar view of a conidium showing the alignment of the setae. Fig. 7. Mature conidium. Fig. 8. *Chaetospermum chaetosporum*. Herb. IMI 95833. Mature conidia. Setae not shown. Fig. 9. *Chaetospermum elasticum* var. *artocarpi*. Herb. IMI 99088. Mature conidia. Polar view of two conidia on the right show the alignment of the setae.



second gathering the dimension of the conidia ranged from  $16-24 \times 3-5 \mu$  with setae  $16-21 \mu$  long

A third gathering on leaves of *Artocarpus integrifolia* differs from the foregoing in the shape of the conidium, position of the setae on the conidium and in the dimensions of the conidia as well as of the setae. Mr Sutton, Assistant Mycologist, Commonwealth Mycological Institute, England considers that these differences fall well within the broad limits of *C. elasticae* Koord. While this treatment is perhaps preferable to naming it as a new species, the author is of the opinion that these characteristics are quite distinct and necessitate the disposal of the collection as a variety of *C. elasticae*.

*Chaetospermum elasticae* Koord var. *artocarpi* var. nov.

Pycnidia foliicola, subepidermalia, innato-erumpentia, mucosa, globosa vel subglobosa, parietibus crassis,  $280-630 \times 140-310 \mu$ . Conidiophori erecti, hyalini, continui, breves. Conidia acrogena, bina vel ternaria vel etiam quaterna, elliptica vel oblongo-elliptica, hyalina, continua,  $15-26 \times 4-6 \mu$ , ad utrumque apicem ternis vel quaternis setis instructa.

Typus lectus in foliis emortuis *Artocarpi integrifoliae*, ad Coffee Research Station, Balehonnur, die 27 augusti 1962, T R Nag Raj, Herb IMI 99088. *Chaetospermum elasticae* Koord var. *artocarpi* var. nov.

Pycnidia foliicole, subepidermal, innato-erumpent, gelatinous, globose to subglobose with thick walls,  $280-630 \times 140-310 \mu$ . Conidiophores erect, hyaline, continuous, short. Conidia acrogenous, in clusters of 2 to 3 or even 4 elliptical to oblong-elliptical, hyaline, continuous,  $15-26 \times 4-6 \mu$  in size, bearing 3 to 4 setae at each end. Setae polar, hyaline,  $9-14 \mu$  long.

On dead rotting leaves of *Artocarpus integrifolia*, Coffee Research Station, Balehonnur, 27-8-1962, T R Nag Raj Type in Herb IMI 99088, Fig 9

Concerning *Chaetospermum camelliae* Agnih

Agnihotbrudu (1962) described this species from a collection made from pruned tea bushes at Tocklai Experiment Station in Assam. The conidia in this species were described as solitary cylindrical conidia,  $16-26 \times 3-6 \mu$  in size, bearing a total of 5-9 setae confined to either end of the spores and  $14-22 \mu$  long. Through the kindness of Mr Sutton, Commonwealth Mycological Institute, England, the author was enabled to examine the material ascribed to this species. The material sent by him comprised of a culture of the fungus on Elm twigs and was labelled '*Chaetospermum camelliae* Agnih

ex type collection' and bore the accn. No. Herb. IMI 82409. The material was compared with the author's collections of *C. elasticae*. The characteristics of the conidia and setae and their dimensions are in agreement with those in *C. elasticae* and for this reason the author is of the opinion that *C. camelliae* is identical with *C. elasticae*.

#### *Conidial development in C. elasticae*

The mode of development of the spore and its setae in *Chaetospermum chaetosporum* was described by Fonseka (1960). A similar study was undertaken by the author on *C. elasticae* from local collections and incidentally on *C. camelliae* from the material received from England. Parallel behaviour was observed in these two species. An account of the developmental stages is presented below and illustrated in Fig. 6, *a-h*.

The conidiophores arise as short erect branches of the fertile hyphae (Fig. 6, *a*). The conidia develop as small papilla at different points on the terminal part of the growing conidiophore. The wall connecting the conidiophore and the conidia is drawn out into short sterigmata and the conidia appear inserted one slightly above the other (Fig. 6, *b, c, d*). An early septum is laid down between the conidia and the conidiophore. While the conidia are growing, the setae appear as short blunt processes (Fig. 6, *d, e, f*) in *C. elasticae* in a subapical position on the outer wall of the conidium and in *C. elasticae* var. *artocarpi* in a polar position. The protoplasm in the young setae seems to be gradually withdrawn and when mature the setae are not stained by cotton blue and acid fuchsin. They are not separated from the conidium by septa. The mature conidia are carried into the central part of the pycnidium by the elongation of the sterigmata (Fig. 6, *g*) and of the conidiophores as well. The conidia are released by the dissolution of the wall at the point of their attachment to the sterigmata on the conidiophores. Under dry conditions the pycnidia shrink in size and the shrinkage is accompanied by irregular ruptures in the wall. When such pycnidia again come in contact with water they swell and the conidia are liberated into the water through the cracks in the wall.

#### SUMMARY

*Chaetospermum chaetosporum* and *C. elasticae* are recorded as new to India. A new variety of *C. elasticae* is described. The author is of the opinion that *C. camelliae* is identical with *C. elasticae*. Conidial development in *C. elasticae* and *C. camelliae* is described and the two species correspond with each other in this feature as well.

## ACKNOWLEDGEMENTS

The author is grateful to Mr Sutton for his help in many ways, and to Dr Santapau, Director Botanical Survey of India, Calcutta, for the Latin diagnosis of the new variety. He is indebted to Dr N G Chokkanna, Director, and Mr K V George, Mycologist, Central Coffee Research Institute, Balehonnur, for laboratory facilities and permission to publish this paper.

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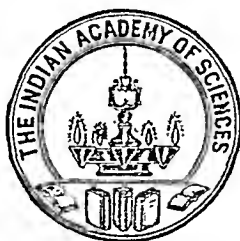
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# LIMNOLOGICAL STUDIES OF TROPICAL IMPOUNDMENTS

## II. Hydrological Features and Plankton of Bhavanisagar Reservoir (Madras State) for 1961-62

BY A. SREENIVASAN, R. SOUNDAR RAJ AND KUMARI FELICY ANTONY  
[*Freshwater Biological Station, Bhavanisagar P.O. (S. India)*]

Received June 27, 1963

(Communicated by Dr. B. S. Bhimachar, F.A.Sc.)

### 1. INTRODUCTION

FISH production from impounded waters is assuming great importance with the ever-increasing need for animal protein. Great deal is therefore to be learnt about the limnology of such impoundments which present peculiar ecological conditions in that the biotope presents partly a lotic and partly a lentic characteristic. Reed and Olive (1956) remarked that very few investigations were made on the biological productivity of sharply fluctuating "Lakes", i.e., impoundments. As reviewed earlier (Sreenivasan, 1963 a) a few accounts are available on impounded waters of temperate climatic countries. Very recently, Harding (1961) published the first report on a tropical impoundment in Africa. Studies on the plankton of Woods Reservoir, Tennessee, were made by Yeatman (1956) while Prowse and Talling (1957) studied the plankton variations and the physico-chemical conditions in a "River lake". Madras State has now a large number of impoundments for irrigation and power generation and based on the successful experience in fish production in Stanley Reservoir, similar programmes have been taken up in other reservoirs also. Schuster *et al.* (1954) have dwelt on the problems involved in fish production from impoundments.

### 2. MATERIAL AND METHODS

Water samples from various depths were collected with a Friedinger sampler twice a month or at least once a month. Analyses were made as enumerated earlier (Sreenivasan, 1963 a). Electrical conductivity was tested by the Dionic water tester and expressed in micro mhos.

### 3. MORPHOMETRIC FEATURES OF THE RESERVOIR

The impoundment is formed by the construction of a dam below the confluence of the rivers Bhavani and Moyar near Pongar village, 20 km.



from Sathvamangalam. The reservoir encloses 30.2 sq miles (19,300 acres) of water spread at full reservoir level. The volume of water at this level is 28,700 m<sup>3</sup> (8127 × 10<sup>6</sup> m<sup>3</sup>). The maximum depth of the reservoir (Z) at FRL is 122 ft (36.9 m). The mean depth (Z<sub>m</sub>) is 37.1 ft (11.3 m). The 'volume development' (Welch 1948) is 1.0 and the 'shore development' 4.0.

#### 4. MICROCLIMATOLOGY OF THE AREA

The daily atmospheric temperature, wind velocity and rainfall (averaged for each month) are presented in Table 1.

TABLE 1 (a)

*Meteorological conditions and data on the reservoir levels of Bhavanisagar Reservoir*

Month (1)	Temperature of air °C		Wind velocity m p h (4)	Reservoir level Ft above M S L		Rainfall mm (6)	Inflow m c ft (7)	Outflow m c ft (8)
	maximum	minimum		maximum	minimum			
	(2)	(3)		(5)	(5)			
January	31.0		1.56	913.9	904.8	16.0	4,382	10,540
February	33.3		Nil	904.5	886.3	101.8	5,177	14,815
March	34.4		3.51	885.5	868.7	Nil	2,811	8,543
April	38.0		3.57	868.5	873.1	1.0	3,164	1,754
May	37.8		5.49	873.2	879.7	79.6	4,624	2,169
June	35.7		4.39	880.0	890.6	68.8	22,520	3,142
July	36.7		2.91	901.6	918.5	40.4	62,470	47,120
August	no data			918.5	918.6	18.5	7,026	8,448
September				918.6	916.8	18.5	7,026	8,448
October				916.6	916.4	189.9	8,872	9,584
November				915.6	916.3	179.4	15,700	14,490
December	29.4		1.90	916.5	910.7	40.4	5,461	9,347

TABLE I (b)

*Meteorological conditions and data on the reservoir levels of Bhavanisagar Reservoir*

1692								
Month (1)	Temperature of air ° C. maximum (2) minimum (3)		Wind velocity m.p.h. (4)	Reservoir level Ft. above M.S.L. maximum (5) minimum		Rainfall mm. (6)	Inflow m.cft. (7)	Outflow m.cft. (8)
January ..	29.4	19.3	2.7	910.4	902.0	4.6	3,922	9,228
February ..	30.8	23.3	1.34	901.8	899.2	192.7	5,151	6,460
March ..	29.0	24.8	1.28	899.0	892.4	3.3	3,947	5,857
April ..	32.7	29.2	2.43	892.1	892.8	32.5	3,338	2,882
May ..	33.8	26.0	3.26	892.9	900.2	89.7	6,530	2,117
June ..	34.0	25.8	4.47	900.3	900.6	27.7	39,020	3,318
July ..	32.7	26.3	6.65	900.5	914.9	22.3	23,240	13,160
August ..	32.3	25.2	5.95	915.0	915.3	83.6	13,980	13,570
September ..	32.9	24.9	3.29	915.3	916.3	50.0	9,826	9,057
October ..	31.0	23.7	1.60	916.2	917.9	218.2	1,991	18,330
November ..	30.8	21.3	0.89	917.9	917.5	43.4	8,156	8,320
December ..	31.0	21.0	0.79	917.6	916.8	191.6	9,638	9,727

## 5. PHYSICO-CHEMICAL CONDITIONS OF THE WATER

The data are represented in Figs. 1-4 but the salient points are presented here.

(i) *Temperature*.—During 1961, two peaks of temperature were noticed, one in March-May (Summer) and the second in November. The highest temperature gradient (4.6° C.) was in November and the lowest in January. This year recorded the lowest surface temperature of 24° C. and lowest bottom temperature of 22.2° C. since the formation of the Reservoir. During the year 1962 also the bimodal temperature maxima were recorded, one in April

and the other in November. The water temperature was lowest in January this year, in contrast to previous years. The maximum difference between the surface and bottom was only  $2.5^{\circ}\text{C}$  noted in October. A feeble thermal stratification was noticeable in July and October. Nearly homothermal conditions were seen during January, August and September.

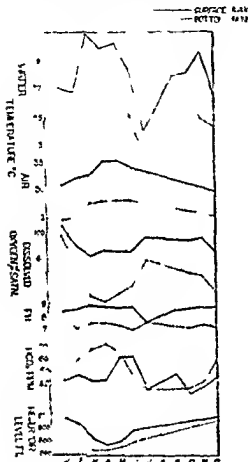


FIG. 1

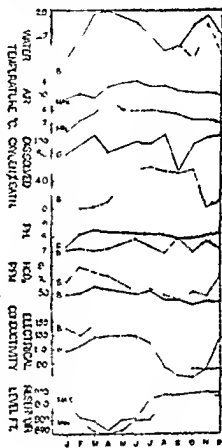


FIG. 2

FIG. 1 2. Fig. 1 Bhavanisagar Reservoir Hydrological Conditions 1961. Fig. 2. Bhavanisagar Reservoir Hydrological Conditions, 1962.

(ii) *Dissolved oxygen*—Following the very high level of supersaturation in December 1960 (Sreenivasan 1963 a) in January 1961 a slight degree of supersaturation of oxygen was noticed. For the rest of the year it did not recur. Severe oxygen deficits were noted from March to June and from October to December 1961.

The surface water was saturated over 50 per cent throughout the year. The trend of severe hypolimnetal oxygen deficits was continued during 1962.

also. In October 1962, very little difference in oxygen concentration between the surface and the bottom was noted. A slight degree of supersaturation was noted in March, August and November–December 1962. The hypolimnion was saturated over 50 per cent. only from July to October. The depth-dissolved oxygen curve (Figs. 3, 4) shows that usually only up to the top 5.0 m. the water was well oxygenated.

(iii) *pH value*.—The variations in pH value were not striking both during 1961 and 1962. The highest pH value of surface-water during 1961 was 8.7 and during 1962 it was 8.5. The lowest pH value of surface-water was in July 1961 (pH 7.5) but during 1962 it was lower still—7.3 in January. pH stratification was common.

(iv) *Carbon dioxide and alkalinity*.—During 1961, free carbon dioxide was present in surface-water only in July whereas it was absent from bottom water only in January. Carbonate alkalinity was a mirror-image of this. The bicarbonate alkalinity increased from January till June in the surface water and then dropped abruptly. In the hypolimnion the drop occurred in June following heavy influx of floodwaters. During 1962, free carbon

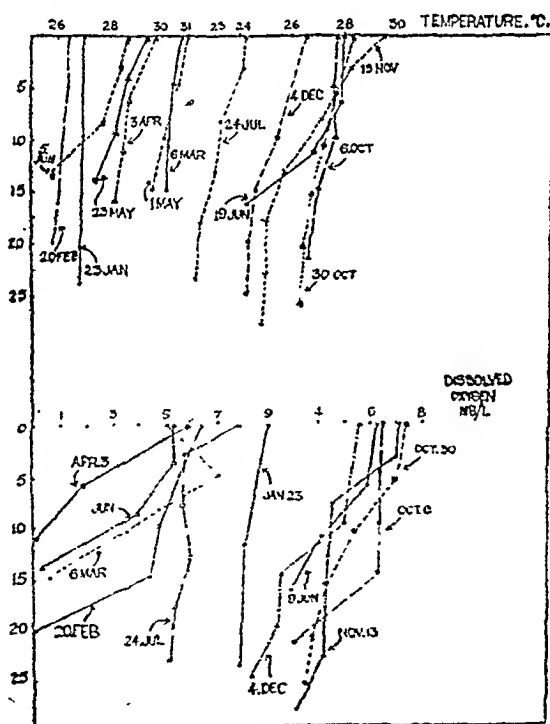


FIG. 3. Temperature and Oxygen-Depth Distribution Curves, 1961.

dioxide was present only in January in surface-water. As in the previous year a fall in bicarbonate alkalinity after floods was noted in July. The bicarbonate alkalinity of hypolimnion was usually higher than that of the epilimnion.

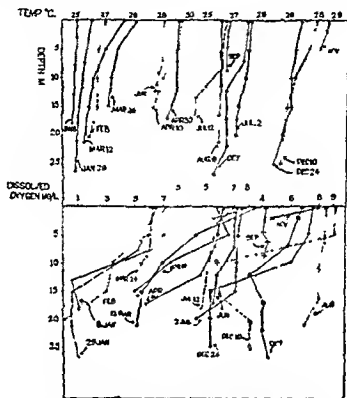


FIG. 4. Temperature and Oxygen-Depth Distribution Curves, 1962.

(v) *Electrical conductivity*.—This is an index of electrolyte content and is proportional to total dissolved solids. The conductivity drops steeply after the July floods. It varied inversely with the reservoir water level. The conductivity was higher in the hypolimnetic water than in surface-water. A positive parallelism also existed between the electrical conductivity and the bicarbonate alkalinity of hypolimnion.

(vi) *Chloride*.—This was rather low—6.0 to 11.0 p.p.m. only and no significant variations were noticed during 1961 and 1962.

(vii) *Hardness*.—This ranged from 40 to 54 p.p.m. throughout 1962.

(viii) *Phosphate, silicate and nitrate*.—Throughout 1961, soluble inorganic phosphate was absent. During 1962, phosphate was detected in quantities of 0.04 to 0.08 p.p.m. in February. At those periods, it occurred

also in the rivers Moyar and Bhavani. In April and June also it was found in the reservoir. At other periods it was absent. In December 'total phosphorus' was present in quantities of 0.208 to 0.225 p.p.m.

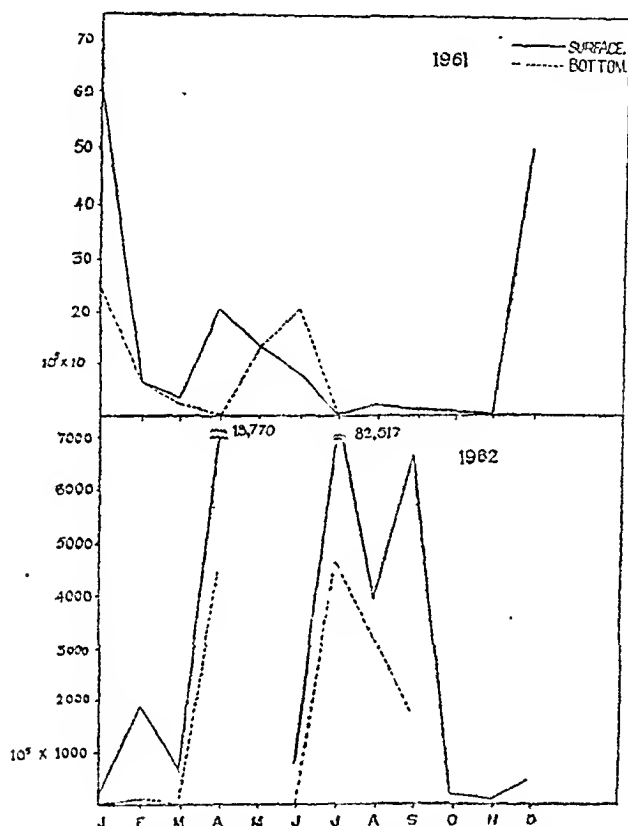


FIG. 5. Phytoplankton (Total) of Bhavanisagar Reservoir.

*Silicate* content varied from 2.0 to 12.0 p.p.m. during 1961. High silicate content was noted from February to June while low quantities were noticed from October to December. During 1962, silicate varied from 0.0 to 15.1 p.p.m. It was present in fairly high quantities from February to May, low from June to October. The highest concentration was in December.

*Nitrate*.—Nitrate was absent throughout the years 1961 and 1962. However, during certain periods nitrite was detected especially below 12 m. depth.

## 6. PLANKTON

Plankton volume was not high and zooplankton was negligible in numbers. During 1961, two peaks of phytoplankton were noted, one in

January and the other in December. But during 1962, the peaks were in March and June. Figure 5 shows the monthly variations in phytoplankton numbers. The occurrence of important species among the phytoplankton is detailed in Fig. 6. A calendar of plankton is furnished in Appendices 1-2. Among the phytoplankters, the dominant groups were the diatoms, blue-green algae and the flagellates. *Nitzschia* and *Melosira* were the ubiquitous diatoms. The more populous among the blue-green algae were *Microcystis* and *Oscillatoria*. *Petridinium* also occurred frequently.

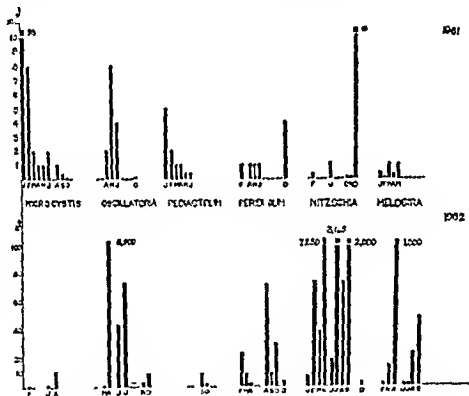


FIG. 6. Dominant Phytoplankton Species of Bhavanisagar Reservoir

## 7 RESERVOIR LEVEL—INFLOW AND OUTFLOW

Lowest storage level in the reservoir was in April which is a period of low inflow, and further, water required for irrigation has been drawn off. From May the reservoir level increases due to greater influx and reduced outflow. The full reservoir level is reached and maintained in December. The fluctuation in water level is not high or sudden. The maximum fluctuation in a month occurred in July 1962 (15 ft). During the storage period of August to December the water level is constant. The inflow-outflow

of water influences the physico-chemical conditions of the water as well as the development of plankton. Maximum inflow is in June, July and August whereas the greater outflow is in July, August and October–November.

## 8. DISCUSSIONS

(a) *Thermal conditions.*—As discussed previously (Sreenivasan, 1963 a), depth variations in temperature were not sharp. Only on two occasions a weak thermal stratification was indicated during 1962. The largest fluctuation between the highest and lowest surface temperature of any year, occurred during 1961— $6.9^{\circ}\text{C}$ . The local air temperature did not influence the thermal conditions of the reservoir during 1961 as in previous years. Only in January 1962 did the lowest temperatures of water and atmosphere coincide. Though the maximum atmospheric temperature occurred in June 1962, the water temperature showed a downward trend. Local wind velocity and rainfall may not explain this but the influx of cool floodwaters from the hills should be the cause, as cited earlier. The high bottom temperature, a characteristic of tropical waters (Ruttner, 1953) lends stability to the water layers and prevents mixing up despite the small temperature difference between layers. This could be seen from the fact that the surface and bottom layers did not completely mix up even during the great floods of July 1961. Only in September 1962 was homothermy noted—this mixing up being due to heavy outflow of cooler water. Withdrawal from bottom prevents stratification (Tarzwell and Palmer, 1951; Vivier, 1960). Reed and Olive (1956) noted that wind broke down even a thermocline of stratum, 43–50 ft.

(b) *Oxygen regimen of the reservoir.*—Compared to the previous five years, oxygen supersaturation was rare during 1961 and 1962. Stratification of oxygen was a common feature and hypolimnial oxygen deficit was severe. Usually, below 5.0 m. a sudden drop in oxygen occurs. Such deficits occurred from February to June and in December of 1961 and from January to April and November–December 1962. The rivers Moyar and Bhavani were better oxygenated and the deficit was less severe in them. Hence it is presumed that the semi-static condition of the water of the reservoir and the oxygen-consuming activity of bottom layers are responsible for this. Further, it is seen that the 'Klinograde' oxygen curve is broken during periods of influx of floodwaters, viz., June, July, August and September 1962 and July 1961. Harding (1961) has shown that in Lake Kariba oxygen deficit was a resultant of thermal stratification. But in our case a semblance of thermal stratification was noted only in July and October 1962 while oxygen deficit was not high during these months. The poor oxygen content of the water during



1961 may be attributed to low level of photosynthesis resulting from heavy influx of floodwaters during this year. This would lead to high 'flushing rate' (Brooks and Woodward, 1965). The 'flushing rate' for the year 1961 was 82 days and for 1962 it was 93 days.

The positioning of the 'intakes' (or discharge sluices) has a bearing on the oxygen regime of the reservoir, as well as that of the river downstream. During summer months—March to May—oxygen depletion occurs in the hypolimnion of the reservoir and hydrogen sulphide is present. The stench of hydrogen sulphide is noticeable near the outlets. The river 'intakes' (sluice) is at a very low level (*plus* 815 ft above M.S.L.) and the discharge carries away the oxygen depleted water. But this did not affect the oxygen content of the river downstream. Oxygen estimations carried out indicate that reaeration takes place within 100 m from the sluice gate—sometimes even at a distance of 20 m the oxygen content is well over 6.0 mg/L (that of the reservoir water at that level being zero). This discharge of de-oxygenated water of the reservoir, also helps to maintain a fair degree of saturation in the epilimnion—preventing possible distress to fishes. Vivier (1960) also found that presence of sluices at lower levels improved the oxygen content of upper layers.

(c) *pH value, alkalinity, etc*—The high pH values reached during previous five years (Sreenivasan, 1963 *a*), was not prevalent during 1961 and 1962. As in the case of dissolved oxygen, reduced photosynthetic activity is the likely reason for this. The pH and alkalinity are comparable to those of Missouri Reservoir and Randalls Reservoir described by Shields (1958). The bicarbonate content was lower during 1961 and 1962 in comparison with previous years. The bicarbonate content of the hypolimnion was usually higher than that of the surface due to tropholytic activities releasing carbon dioxide. Though oxygen deficit in the hypolimnion is used as a measure of productivity (Thienemann 1928, Saha 1959), Ohle (1952) feels hypolimnial accumulation of carbon dioxide is a better index. The foregoing discussion supports this view. Hutchinson (1957) opines that vertical distribution of carbon dioxide reflects in a way the vertical distribution of oxygen deficit. Yeatman (1956) also noted that at depths the water will be less alkaline or neutral or acid because of the carbon dioxide resulting from respiration of zoo plankton and that produced by decomposing activities. The low hardness of the water is due to low quantities of sulphates, and chlorides of calcium magnesium as well as that of alkali metals. The lower concentration of carbonates and bicarbonates during and following the floods indicates that these are not leached out from the drainage basin and must

have been produced by decomposers in the hypolimnion. Electrical conductivity decreased after-floods confirming this opinion. The alkalinity, hardness and electrical conductivity of Bhavanisagar Reservoir was less than that of Stanley Reservoir (Sreenivasan, 1963 *b*). Edmondson (1956) noted a distinct increase in conductivity in the bottom, as in our case (Fig. 2). He feels the change in conductivity in a way permits useful interpretations in limnology.

(*d*) *Other constituents*.—Nitrate was absent at all times examined at all depths during 1961–62. Soluble inorganic phosphate was absent throughout 1961 but during 1962 it was present during February, April and June. In December, “Total phosphate” was present in quantities ranging from 0.208 to 0.225 p.p.m. As observed earlier (Sreenivasan, 1963 *a, b*) absence of phosphate or poverty in these tropical reservoirs is a very notable feature. Clearly, the rivers do not bring any phosphates nor does the bottom ooze release any into the over-laying waters. Ruttner (1953) makes it clear that ‘no direct relationship exists between the quantity of total plankton or of individual species present at any one moment and the content of nutrients measurable at the same time....’. From the point of view of providing drinking water supply this fact may be favourable to sanitarians (Tarzwell and Palmer, 1951), but as far as fish production is concerned this is unfavourable. Rapid turnover of the meagre phosphorous resources is assumed since very little is left in the water for estimation. An interesting feature is the appearance of nitrite in intermediary layers. It is not clear whether this is the result of nitrification or reduction of nitrate but considering the low oxygen content of these layers, the latter seems to be more probable.

(*e*) *Plankton*.—Quantitatively, the volume of net plankton in this reservoir is almost negligible—less than 0.05 ml./10 L. The poor development of plankton may partly be due to edaphic conditions and partly due to continuous inflow and outflow of water. There was no correlation between temperature and plankton numbers, which supports Talling’s view (1957) that temperature is unlikely to modify directly the distribution of phytoplankton. The peaks of plankton numbers occur, at different periods during the two years. The major peak occurred in January in 1961 but in 1962 it was during July. Phytoplankton was more abundant during 1962 than during 1961. The virtual absence of phytoplankton from July to November 1961 might be due to excessive floods.

The seasonal abundance of six dominant species is indicated in Fig. 6. These also show no uniform pattern from year to year. During 1961, *Microcystis* was present in large numbers only in January–February,

the cooler months whereas during 1962 its abundance was noted only in August. *Oscillatoria*, the other blue green alga, reached a peak in May 1961 but during 1962, it was in April. Diatom peak, with dominance of *Nitzschia*, was reached in December 1961 but during 1962 it was abundant for the major part of the year except October–November. April 1962 witnessed an outburst of *Nitzschia* and *Melosira* but this did not lead to supersaturation of oxygen. *Pediastrum* had its peak in January decreased subsequently and was not recorded after June 1961. In 1962 its peak was noted in October. *Peridinium* occurred sporadically both during 1961 and 1962. Supersaturation of oxygen occurred during November–December 1962 though phytoplankton was not abundant. Griffith (1955) also did not find any correlation between oxygen saturation and phytoplankton. Seasonal changes in phytoplankton communities have been noted by Hartman and Graffins (1960) in Pymatuning reservoir.

The edaphic factors, viz., the basic soil type (*vide* Sreenivasan, 1963 a) are unfavourable for high biological productivity (Tarzwell and Palmer, 1951). Brook and Woodward (1956) think that only rapidly reproducing species such as *Synedra*, *Cyclotella*, *Chroocoonas*, *Dinobryon*, etc., can succeed in reservoirs with continuous inflow-outflow. But, in Bhavanisagar Reservoir, we find that these occur rarely whereas certain blue-green algae and diatoms occur more often. Plankton develop mainly in the surface-water of the open reservoir and in littoral areas since these are practically undisturbed, the outflow occurring through the low-level discharges (Kimsey, 1958).

Talling (1957) found that silicate content of Lake Victoria varied from 7–11 mg/L, somewhat similar to our results. He also noted a correlation between phytoplankton and slowing down of current. The high numbers of *Nitzschia* during December 1961 coincided with low silicate levels. Prowse and Talling (1958) in their work on a Nile 'river lake' found that *Melosira* was the only alga likely to cause depletion of silica. The occurrence of total phosphorus in November–December 1962 coincides with poor phytoplankton population. Possibly, decay of the plankton had released phosphates. Yeatman (1956) thinks that temperature is one of the chief external factors influencing seasonal succession but this is not true in Bhavanisagar Reservoir. High phytoplankton populations especially of *Myxophyceae*, were noted in an impoundment in Pennsylvania by Tryon and Jackson (1952). Similar situation has not yet arisen in Bhavanisagar Reservoir.

(f) *Physical features*—This reservoir has a volume development of 1.0 but this is shallower (mean depth 11.3 m) than some other reservoirs like Mettur and Amaravathy. This might increase the euphotic zone and

thereby the photosynthesis. Primary production experiments (Sreenivasan, unpublished) shows the compensation depth to be invariably not less than 5.0 m. However, the wave action and the fluctuating water levels practically create a littoral desert—absence of vegetation or bottom fauna or “aufwuchs” community in the littoral zone. Edaphic factors are none too favourable for productivity. Except for a month or two after floods, the water is clear and light penetration is good (Seschi disc visibility not less than 1.0 m.). The inflow-outflow rates influence the physico-chemical conditions and plankton abundance. Compared to the years 1959 and 1960, when the “flushing rate” and “replacement quotient” were over 120 days, during 1961 they were 82 days and 76 days respectively. This did not permit large increase in plankton. During 1962, when the “replacement quotient” and “flushing rate” were 117 days and 93 days respectively, the plankton was more abundant than during 1961. In fact from June to November 1961 the phytoplankton was negligible. During the three months, June to August, 2/3rd of the entire years’ inflow and 46 per cent. of the years’ outflow took place. The more pronounced effect of the influx of floodwaters from June to August was to reduce the water temperature, decrease the methyl orange alkalinity (bicarbonate) and the electrical conductivity. It also reduced the oxygen deficit in the hypolimnion by mixing up the layers. Ruttner (1953) feels that inflow-outflow is one of the most radical depletion factors carrying away a part of the plankton and causing impoverishment of the strata concerned. The closure of the canal sluice (level *plus* 845 ft.) from April to June leads to severe oxygen depletion but its effects on temperature is not so manifest. Chalupa and Cervenka (1958) also noted that “contrary to lakes, in reservoirs, specific conditions are established where changes cannot be expected as in temperature regimen of lakes”. The findings of these authors on the thermal and oxygen regimen of Sedlice Reservoir in Czechoslovakia almost resemble the conditions noticeable in Bhavanisagar Reservoir. Drachev (1962) in Russia found that oxygen consumption increased with a decrease in the discharge of water into the river which in turn coincided with the inflow rate. This is true also in Bhavanisagar Reservoir. However, his remarks that winds are of predominant importance in mixing up in the reservoirs is not valid for our reservoir. Tropical reservoirs seem to be a separate class differing in many respects from impoundments in temperate regions. Further studies on such reservoirs is warranted.

#### SUMMARY

The hydrological conditions of the Bhavanisagar Reservoir and the plankton for the years 1961 and 1962 are detailed. There seems to be some

difference in physico-chemical conditions during these two years when compared with the data of previous five years. Thermal stratification was rare but oxygen deficit in hypolimnion was severe. Supersaturation was rare and the higher pH values noted between 1956-1960 were not recorded during 1961-62. The electrical conductivity increased with depth and showed seasonal changes paralleling the changes in bicarbonate content. Deficiency of nutrients, phosphate and nitrate continued as usual though the silicate content was normal. The plankton organisms occurring in different months are listed. Excessive inflow and outflow restricted plankton blooms. The effect of the position of discharge sluices on the oxygen regimen and temperature are discussed.

## 10 ACKNOWLEDGEMENTS

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## APPENDIX 1

### *Phytoplankton Calendar of Bhavanisagar Reservoir*

1961

#### CYANOPHYCEAE

<i>Micracystis</i>	January to October
<i>Oscillatoria</i>	April-June August-October
<i>Merismopedia</i>	June, August, September, December
<i>Anabaena</i>	April-May
<i>Microcoleus</i>	February, May-June
<i>Spirulina</i>	June

#### CHLOROPHYCEAE

<i>Pediastrum</i>	January-June, October-November.
<i>Oedogonium</i>	June
<i>Eudarina</i>	August

#### DESMIDS

<i>Staurastrum</i>	. January, May-June, December.
<i>Casmarium</i>	. August, December.

#### FLAGELLATA

<i>Peridinium</i>	. February, April-June, September, December.
<i>Trachelomonas</i>	. August, November.

#### DIATOMAE

<i>Synedra</i>	.. August-October.
<i>Navicula</i>	.. August-October.
<i>Nitzschia</i>	. February, June-July, September-December.
<i>Melosira</i>	.. January, February, April-June, December.
<i>Pleurosigma</i>	.. June
<i>Amphora</i>	.. August.
<i>Cyclotella</i>	.. September, December.
<i>Gomphorema</i>	.. October.
<i>Fragilaria</i>	.. October.

1962

## CYANOPHYCEAE

- Microcystis* .. January–February, June, August, November.  
*Oscillatoria* .. February–April, June, August, November.  
*Merismopedia* .. February, June, August–December.  
*Microcoleus* .. February, April, July, November.  
*Holopedium* .. July, September, December.  
*Cylindrospermum* .. July.  
*Anabaena* .. September, December.

## CHLOROPHYCEAE

- Pediastrum* .. July–October, December.  
*Dictyosphaerium* .. August.

## DESMID

- Staurostrum* .. June, July, September–December.  
*Cosmarium* .. February, July–September, December.  
*Ankistrodesmus* .. January.  
*Scenedesmus* .. September.  
*Closterium* .. September.  
*Euastrum* .. December.

## FLAGELLATA

- Peridinium* .. February, March, July–December.  
*Trachelomonas* .. March, July–August.

## DIATOMS

- Nitzschia* .. June–September, November–December.  
*Navicula* .. January, March–April, June–October, December.  
*Melosira* .. February–April, June–September, December.  
*Stephanodiscus* .. February, June–July.  
*Synedra* .. March, June, December.  
*Cyclotella* .. July–September, December.



## APPENDIX 2

### *Zooplankton Calendar of Bhavanisagar Reservoir*

1961

#### PROTOZOANS

*Diffugia* February-March, May-June

#### ROTIFERS

*Brachionus* January-August.

*Diurella* February, May-June, August.

*Anuraea* February, July.

*Asplanchna* August

*Monostyla* January-May

*Floscularia* March-June

*Polyarthra* .. February-July.

*Triarthra* .. July.

*Hydatina* .. May

#### CRUSTACEAN

*Nauplius* .. February, May-July.

*Copepod* .. January-October.

*Cladocera* .. February, June-August, October

1962

#### PROTOZOANS

*Diffugia* .. April.

*Coleps* .. September.

#### ROTIFERS

*Brachionus* .. April, August-October.

*Diurella* .. February, June, August.

*Anuraea* . February, April, August-September.

*Pterodina* .. March-April

*Asplanchna* .. April

<i>Floscularia</i>	.. February, March, June, August–October.
<i>Polyarthra</i>	.. February, April, August–October.
<i>Triarthra</i>	.. August.
<i>Tetramastix</i>	.. April.
<i>Anapus</i>	.. September.

CRUSTACEANS

<i>Nauplius</i>	.. February–March, August–October.
<i>Copepod</i>	.. April, July–August, October.
<i>Cladocera</i>	.. July–August, October.

# CHROMOSOME NUMBERS IN SOME MEDICINAL COMPOSITES

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SOME cytological work has been done in medicinal Composites growing in different parts of India, but very little work has been done on the plants growing in Jammu and Kashmir areas. It is now an established fact that in nature varieties with different chromosome numbers occur in the same species of plants. In the medicinal plants such geographical races are also bound to show differences in their drug content, so, a cytological survey has been undertaken for some of the medicinal Composites growing in J and K State. Cytological observations have been made mostly from pollen mother cells and root-tip cells. In some, leaf squashes were also used. Materials were fixed in 1:3 Acetic alcohol mostly, but in some, a modified fixative was also utilized. Preparations were made permanent by using N-Butyl-alcohol Acetic acid series and mounted in Euparal. All drawings were made with the help of a camera lucida, at the level of Table and from the permanent preparations only. Chromosome numbers of the plants marked \* are reported for the first time, while in others the previously reported chromosome numbers have been confirmed.

The chromosome number of medicinal plants studied is given in Table I.

A diploid *Artemisia vulgaris* ( $2n = 18$  and  $n = 9$ ) has been found in North-Western Himalayas, where till now tetraploid and hexaploid forms were believed to exist abundantly. *A. vulgaris* got from Germany possesses  $n = 8$  and  $2n = 16$  chromosome numbers. The present studies corroborate the views of Khoshoo and Sobti (1958) regarding the presence of two basic numbers for the genus, viz.,  $n = 8$  and  $n = 9$ . *Erigeron multicaulis* with  $n = 27$  and  $2n = 54$  chromosome numbers appears to be a hexaploid. The genus *Taraxacum* is regarded as a complex polyploid apomictic with a basic number  $x = 8$  by Darlington and Janaki Ammal (1945) and Darlington and

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TABLE I

Sl. No.	Name	Collected from	Chromosome number		Ploidy	Previous report
			<i>np</i>	<i>2n</i>		
1	<i>Ageratum conyzoides</i> Linn. (Srinagar Type)	.. Srinagar	10	20	Diploid	$2n = 20$ and 40
	(Jammu Type)	.. Jammu	10	20	Do.	
2	<i>Artemisia absinthium</i> Linn.	.. Srinagar	9	18	Do.	$2n = 18$
3	<i>Artemisia vestita</i> Wall	.. Jammu (RNC Garden)	9	18	Do	$2n = 18$
4*	<i>Artemisia vulgaris</i> Linn.	.. Do.	9	18	Do.	$2n = 18, 36, 54$
5	<i>Artemisia vulgaris</i> Linn. (Germany)	Do.	8	16	Do.	$2n = 16$
6	<i>Chrysanthemum cinerariifolium</i> viss.	.. Srinagar	9	18	Do.	$2n = 18$
7	<i>Cichorium intybus</i> Linn.	.. Do.	9	18	Do.	$2n = 18$
8*	<i>Cousinia microcarpa</i>	.. Do.	13	26	Do.	
9*	<i>Cythocline lyrata</i> Case	.. Jammu (RNC Garden)	11	22	Do.	
10*	<i>Echinops echinatus</i>	.. Jammu	14	28	Do.	
11*	<i>Erigeron multicaulis</i> Wall	.. Srinagar	27	54	Hexaploid	
12	<i>Galansoga parviflora</i> Cav.	.. Do.	8	16	Diploid	$2n = 16$
13	<i>Matricaria chamomilla</i> (a) Jammu Type	.. Jammu (RNC Garden)	9	18	Do.	$2n = 18$
	(b) Srinagar Type	.. Srinagar	9	18	Do.	$2n = 18$
14	<i>Notonia grandiflora</i> Dc.	.. Jammu (RNC Garden)	10	20	Do.	$2n = 20$
15*	<i>Siegesbeckia orientalis</i>	.. Srinagar	15	30	Do.	
16	<i>Souchus asper</i> Linn.	.. Do.	9	18	Do.	$2n = 28$
17	<i>Souchus oleraceus</i> Linn.	.. Jammu	16	32		$2n = 32$
18*	<i>Taraxacum officinale</i> Webb	.. Kud	12	24		$2n = 16, 18, 24, 32, 34, 36, 38$
19	<i>Vernonia cinerea</i> Less	.. Jammu	9	18	Do.	$2n = 18$
20	<i>Weddelia calandulaceae</i> Less	.. Jammu (RNC Garden)	25	50		$2n = 50$

Note:—(RNC Garden) = Ram Nath Chopra Garden of Medicinal Plants,

Wylie (1955) But in *T. officinalis*, Panigrahi and Kamathy (1960) have recorded four bivalents in meiosis during diakinesis stage. These results were further confirmed by Sharma and Chatterjee (1961) who reported  $2n=8$ , from the material collected from Khasi Hills. So they assign a much lower basic chromosome number for the genus, i.e.,  $x=4$  in contradiction with the previously assigned number  $x=8$ . In this plant, seed-setting is reported to be profuse (Sharma and Chatterjee, 1961). In the present studies the



TEXT FIGS. 1-15 Fig. 1 First Metastene metaphase of *Matricaria chamomilla*,  $n=9$ ,  $\times 1,900$ . Fig. 2 Diakinesis of *Cythocline lyrata*,  $n=11$ ,  $\times 1,900$ . Fig. 3 Diakinesis of *Sonchus oleraceus*,  $n=16$ ,  $\times 2,400$ . Fig. 4 Late diplotene of *Galinsoga parviflora*,  $n=8$ ,  $\times 2,400$ . Fig. 5 Diakinesis of *Wedelia calendulaceae*,  $n=25$ ,  $\times 2,400$ . Fig. 6 Diakinesis of *Erigeron multicaulis*,  $n=27$ ,  $\times 3,000$ . Fig. 7 Diplotene stage of *Ageratum conyzoides*,  $n=10$ ,  $\times 2,400$ . Fig. 8 Diakinesis of *Artemisia roxburghiana*,  $n=8$ ,  $\times 1,800$ . Fig. 9 Diakinesis of *Artemisia vulgaris*,  $n=9$ ,  $\times 1,900$ . Fig. 10 Diakinesis of *Artemisia verba*,  $n=9$ ,  $\times 2,400$ . Fig. 11 Diakinesis of *Cleistanthus luteus*,  $n=9$ ,  $\times 1,900$ . Fig. 12 Diakinesis of *Cassia microcarpa*,  $n=13$ ,  $\times 1,900$ . Fig. 13 Late diplotene stage of *Lemmonia chinensis*,  $n=9$ ,  $\times 1,900$ . Fig. 14 Diakinesis of *Sieversbeckia orientalis*,  $n=15$ ,  $\times 2,400$ .

material collected from Kud (J and K State) showed  $n = 12$  and  $2n = 24$ . These findings are in conformity with those of Furnkranz (1960), who has reported a variable number for the species ranging from  $2n = 16$  to 37. This *Taraxacum* does not produce viable seeds but is burdened with the expenses of pollen production. Here replacement of sexual reproduction by apomixis has taken place. Since there is some pairing and consequent crossing over, there is restricted recombination which accounts for its limited but sporadic instability resulting in the production of diverse morphological, ecological and cytological variants. In this population strains with 24, 23 and 22 chromosomes have been occasionally found.

Ishikawa (1911) from tropics reported diploid forms of *Ageratum conyzoides* while Mitra (1947) from the material collected from Calcutta found 20 bivalents at diakinesis, and thus found tetraploid forms of the plant for the first time. Under the present investigations only the diploid forms of the plant have been found for the plants growing wild in both Jammu and Kashmir area with chromosome number  $n = 10$  and  $2n = 18-20$ . *Notonia grandiflora* from a study of meiosis appears to be a structural hybrid involving all sorts of chromosomal abnormalities leading to the failure of viable seed production resulting in a loss of fertility and consequent replacement of sexual reproduction by an asexual reproduction. Complete details of its cytogenetics and that of the *Taraxacum officinale* will be communicated very shortly. Genus *Sonchus* is characterised by an oval to elliptical nucleolus as revealed by a study of meiosis. From this survey of the chromosome numbers it appears that the majority of Composites have evolved from a basic number 8 and 9. The species that have retained this number possess large, long and conspicuous chromosomes while plants with high chromosome numbers have small chromosomes and as the chromosome number increases from its basic number, the relative size of the individual chromosome shows a proportionate decrease. There is every likelihood that the plants with a high chromosome number and small-sized chromosomes, polyploidy, fragmentation with either deletion or addition of an acentric or centric fragment may have played a major role in the evolution and speciation of Composites.

#### SUMMARY

A survey of the chromosome numbers has been conducted in 20 species of medicinally important Composites of Jammu and Kashmir State distributed under 16 genera.

## ACKNOWLEDGEMENTS

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My thanks are due to Dr I C Chopra Deputy Director, Regional Research Laboratory Jammu for providing the laboratory facilities

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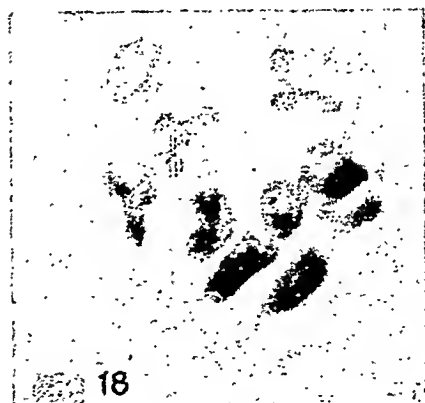
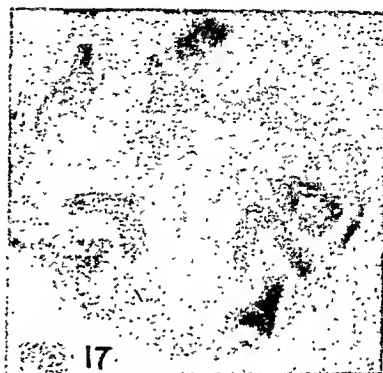
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\*Not consulted in original

## EXPLANATION OF PLATE I

- FIG 16 Diakinesis of *Chrysanthemum cinerariifolium* showing nine bivalents, out of which one bivalent has just separated apart one bivalent is nucleolar  $\times 2,100$ .
- FIG 17 Diakinesis of *Arenaria ciliolata* showing nine bivalents with one nucleolar bivalent  $\times 2,100$ .
- FIG 18 Diakinesis of *metula grandiflora* with one nucleolar bivalent,  $\times 2,100$



FIGS. 16-18



# A NEW SPECIES OF *PALMOXYLON* FROM THE DECCAN INTERTRAPPEAN BEDS

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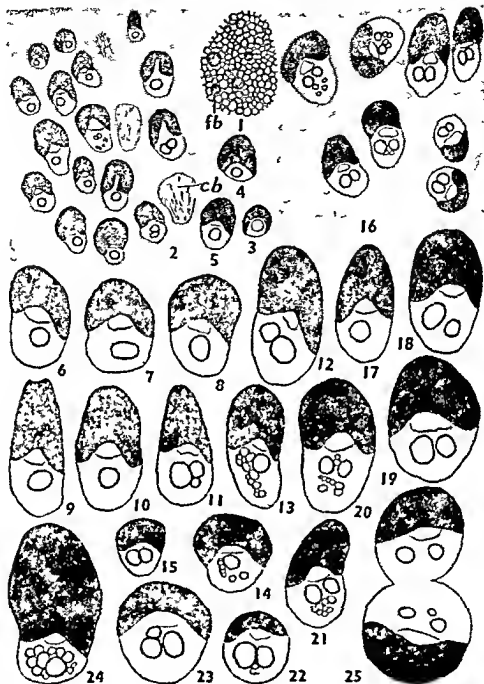
## INTRODUCTION

THE specimen described here is one of the many fragments of silicified palm woods collected from Mohgaon Kalan area, Madhya Pradesh, a locality from which a number of petrified palm woods have been described. The specimen is fragmentary but measuring about 6.5 cm. in length and 3.4 cm. in breadth. The preservation is not quite satisfactory, yet the arrangement of the bundles in the different zones of the wood is very clear to the naked eye on the polished surface of the specimen. The specimen is brownish-yellow in colour. In thin sections the yellow portions look white.

## ANATOMICAL DESCRIPTIONS

Transverse sections of the specimen show three distinct zones—the Dermal, Sub-dermal and Central (Plate II, Photo 1). Fortunately, a small portion of the cortex also is preserved on the outside of these zones. Thin sections of the cortical region show mainly fibrous bundles. They are of varying sizes and irregularly distributed (Fig. 1). The ground tissue is compactly arranged with thin-walled cells.

*Dermal zone* consists of fibrovascular bundles regularly orientated and closely placed (Fig. 2; Plate II, Photo 2). The row of bundles just below the cortex is very small in size and are more or less round to oval in shape (Figs. 3–5). The rest of the bundles are elongated (Figs. 6–13). In between these elongated bundles are seen some smaller bundles (Figs. 14 and 15). The frequency of the bundles is 245 to 320 per cm.<sup>2</sup> and they are about 0.2–0.5 mm. in diameter. The bundles are very crowded, so that they leave very little space for the ground tissue. And even this is very compact in nature with some intercellular spaces. The *f/v* ratio in the main bundles is about 0.5/1–1.4/1. The auricular lobes are round and the median sinus is concave. Phloem is preserved in some of the bundles. The vascular part consists mostly of



FIGS. 1-25 Fig. 1 Transverse section of the cortical region showing the fibro-bundles and the ground tissue,  $\times 56$ . fb, fibrous bundle. Fig. 2 Distribution of the fibrovascular bundles in the dermal zone,  $\times 36$ . cb, crushed bundle. Figs. 3-5 Smaller bundles in the region below the cortex,  $\times 60$ . Figs. 6-15 Different kinds of bundles in the dermal zone,  $\times 60$ . Fig. 16 Distribution of the fibrovascular bundles in the sub-dermal zone,  $\times 36$ . Figs. 17-23 Different kinds of bundles in the sub-dermal zone,  $\times 60$ . Fig. 24 One fibrovascular bundle of the sub-dermal zone showing the grouped xylem vessels,  $\times 60$ . Fig. 25 One lined bundle showing the fusion on the vascular part,  $\times 60$ .

one main xylem vessel (Figs. 6-10) but sometimes a pair of vessels lying side by side (Figs. 11 and 12). Protoxylem is present. The vessels are completely excluded or are outside the median sinus. There is no radiating or tabular parenchyma seen around the fibrovascular bundles.

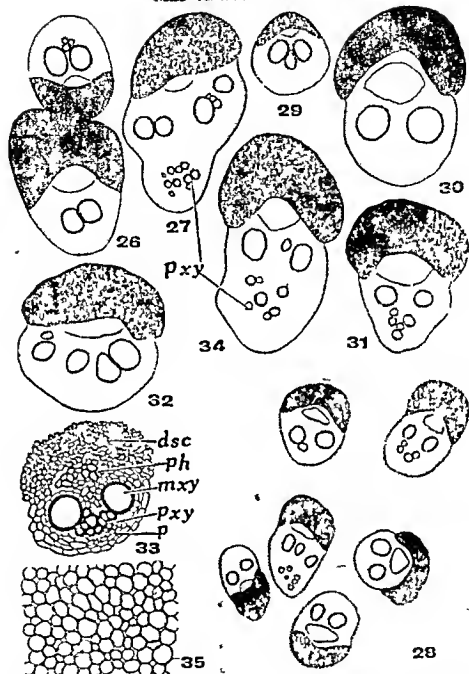
*Sub-dermal zone.*—The majority of the fibrovascular bundles are regularly orientated and are less densely crowded (Fig. 16; Plate II, Photo 3). They are 150-170 per cm.<sup>2</sup> and are of different sizes and shapes (Figs. 17-23). The bundles are 0.3-0.6 mm. in diameter and their  $f/v$  ratio varies from 0.4/1-1/1. The auricular lobes are mostly round, the median sinus is concave to somewhat flat (Figs. 17-23). Usually, there are two big, circular to oval main vessels, excluded, placed side by side in the vascular parts of the bundles (Figs. 18-23). Some of the bundles consist of a number of xylem vessels grouped together (Fig. 24). This type of bundles are seen in all the three zones. Smaller rounded bundles occur in between the other main bundles (Fig. 22). Radiating and tabular parenchyma cells surrounding the bundles are totally absent. Here and there are found some fused bundles. Their fusion takes place either in the sclerenchyma parts or in the vascular parts (Figs. 25 and 26). Leaf-traces are often found in this zone. They are bigger than the main bundles (Fig. 27).

*Central zone* is distinguished by the irregular orientation of the fibrovascular bundles (Fig. 28; Plate II, Photo 4) and they are far apart from each other. They are 80-125 per cm.<sup>2</sup> and are 0.5-0.8 mm. in diameter. The varying form of bundles is seen in Figs. 29-33. The  $f/v$  ratio of the bundles is 0.3/1-0.8/1. The median sinus is somewhat concave and auricular lobes are round. Rounded, excluded, paired vessels occur more commonly in the vascular parts of the bundles (Figs. 30, 31 and 33; Plate III, Photo 5). In between the big bundles there occur occasionally a few small fibrovascular bundles. Radiating or tabular parenchyma cells are conspicuous by their absence. Leaf-trace bundles are very common here (Fig. 34).

*Fibrous bundles* are present only in the cortex. They are altogether absent in the other three zones.

*Stigmata* also have not been observed in any of the fibrovascular bundles.

*Phloem* is preserved in most of the bundles in all the three zones. A more or less triangular-shaped phloem cavity is clearly seen in the bundles in which the phloem is not preserved.



FIGS. 26-35 Fig. 26. One fused bundle showing the fusion on the sclerenchyma part,  $\times 75$  Fig. 27. One leaf trace bundle of the sub-dermal zone,  $\times 75$  Pxy, protoxylem Fig. 28. Distribution of the bundles in the central zone,  $\times 45$  Fig. 29-32. Different types of bundles in the central zone,  $\times 75$  Fig. 33. One fibrovascular bundle of the central zone showing all the tissues,  $\times 75$ , dsc, dorsal sclerenchyma, mxy, metaxylem, P, parenchyma, ph, phloem, pxy, protoxylem. Fig. 34. One leaf-trace bundle of the central zone,  $\times 75$  pxy, protoxylem. Fig. 35. A portion of the ground tissue in the central zone,  $\times 75$

The ground tissue is compact throughout the wood (Fig. 35). The cells are thin-walled, oval to rounded in shape with slight intercellular spaces occasionally seen in between them. In the longitudinal sections these cells are seen to be placed contiguously in vertical rows (Plate III, Photo 6) as in other palm woods. There are some special type of cells present in the ground tissue (Plate II, Photos 2-4). The bad preservation makes it difficult to say what they are. They are mostly single-celled structures, but sometimes a chain of five or six cells joined together are also seen. Some of them show bar-like thickenings. The nature of these cells could not be determined. But they may be comparable to idioblasts as reported in the case of *Palmoxyton sclerodermum* by Sahni (1943).

Longitudinal sections show the pitting of the xylem vessels is of the multiseriate scalariform as well as typical helical types (Plate III, Photo 6). The perforated end-walls are obliquely placed and show a series of widely spaced parallel bars of thickening (Plate III, Photo 7).

#### DISCUSSION

Quite a large number of palm woods have been described from the Deccan Intertrappean beds by several authors (Sahni, 1931, 1943, 1946; Rode, 1933; Shukla, 1939, 1946; Ramanujam, 1953, 1958; Lakhanpal, 1955; Mahabale, 1958; Prakash, 1958, 1961; Rao and Menon, 1962, 1963). The characters of all these palms have been incorporated in a tabular form (Rao and Menon, 1963). The specimen described in the present paper shares some character or other with each one of the species in Table I. The absence or presence of fibrous bundles, stigmata, radiating or tabular parenchyma, posterior sclerenchyma, are some such characters—which may be considered as comparatively unimportant. But a closer comparison can be possible with *Palmoxyton puratanam*, *Palmoxyton narayanai* and *Palmoxyton maheshwarii*. The specimen described in this paper resembles the above-mentioned three species in a few of the characters, but differs from them in some of the important characters, like frequency of the bundles in the various zones of the wood;  $f/v$  ratio of the bundles; and the diameter and nature of the bundles. The number of bundles per  $\text{cm}^2$  in this species is greater than in *P. puratanam*, *P. narayanai* and the bundles in the dermal zone of *P. maheshwarii* but lesser than those in the sub-dermal and central zones. The  $f/v$  ratio of *P. puratanam* and *P. narayanai* is greater than the  $f/v$  ratio of the specimen described here, while  $f/v$  ratio of *P. maheshwarii* is lesser. As far as the nature, shape and type of the fibrovascular bundles are concerned these four species are quite different from each other. This can be noted easily from the figures and

TABLE

No.	Name of the <i>Palmyroxylon</i> species	Cortex	Frequency of the bundles	f/v ratio of the bundles	Diameter of the bundles (in mm.)	Base of the dorsal sclerenchyma
1	<i>Palmyroxylon puratanam</i> (Ramanujam C. G. K., 1958)	Imperfectly preserved thin-walled parenchyma cells. Numerous fibrous bundles of various shapes and sizes. Some structures like scleroids or murilloge canals present. A few normal fibrovascular bundles are seen.	D-80-100/cm. <sup>2</sup> SD-65-75/cm. <sup>2</sup> C-20-25/cm. <sup>2</sup>	D-4/1 SD-3/2-1 1/1 C-1 1/2/1	D-0.8-0.5 SD-0.05-1.2 C-1.1-1.5	Rounded cordate median sinus. Sometimes reniform median sinus.
2	<i>Palmyroxylon narayanai</i> (Rao & Menon, 1962)	Not preserved	D-105-110/cm. <sup>2</sup> SD-60-66/cm. <sup>2</sup> C-40-45/cm. <sup>2</sup>	D-1.3/1-4/1 SD-1/1-2/1 C-0.6/1-1.7/1	D-0.25-0.41 SD-0.17-0.37 C-0.19-0.31	Flat median sinus, auricular lobes somewhat round.
3	<i>Palmyroxylon maheshwarii</i> (Rao & Menon, 1963)	Consists of both fibrovascular and fibrous bundles. Fibrovascular bundles are elongated. The f/v ratio is 3/1-10.5/1 and diameter C-14 to 0.42 mm. Vertical sclerenchyma present in some bundles. Frequency of the bundles is 60-55/cm. <sup>2</sup>	D-540-650/cm. <sup>2</sup> SD-66-100/cm. <sup>2</sup> C-35-45/cm. <sup>2</sup>	D-0.2/1-0.6/1 SD-0.12/1-0.3/1 C-0.1/1-0.2/1	D-0.12-0.23 SD-0.23-0.43 C-0.17-0.56	Crescent type of median sinus.
4	<i>Palmyroxylon sikani</i> (Menon, 1963)	Preserved only a small portion. Only fibrous bundles are present. Ground parenchyma consists of thin-walled cells.	D-245-320/cm. <sup>2</sup> SD-150-170/cm. <sup>2</sup> C-80-125/cm. <sup>2</sup>	D-0.5/1-1.4/1 SD-0.4/1-1/1 C-0.3/1-0.8/1	D-0.2-0.5 SD-0.3-0.6 C-0.5-0.8	Concave to flat median sinus and round auricular lobes.

D, Dermal zone; SD, Sub-dermal zone; C, Central zone.

photographs of the species. Except in *P. narayanai* the cortex is preserved in all the other three species. It is more or less of the same type in *P. puratanam* and the species described here. In *P. maheshwarii* the fibrovascular bundles of the cortex are quite elongated and the fibrous part is three to ten times

I

Phloem	Xylem vessels	Radiating and tabular parenchyma	Fibrous bundles	Stegmata	Posterior sclerenchyma	Leaf-trace bundles	Ground tissue
Present in some of the bundles	D-1 SD-2 C-2 Protoxylem present	2-layered tabular parenchyma present but not radiating	Absent	Absent	Found in the leaf-trace bundles	Present	Compact thick-walled parenchymatous cells. They are angular and do not show any lobes or processes
Not preserved	D-2 Xylem vessels side by side SD-2.4 C-3.4	Radiating parenchyma around the leaf-trace bundles but not tabular parenchyma	Absent	Absent	Absent	Present	Closely placed thin-walled cells of various shapes
Absent	D-1-2 side by side SD-2 C-2 Protoxylem present	Absent	Present in cortex but absent in the other zones	Absent	Present only in leaf-trace bundles	Present	Thin-walled round cells overlapping each other and having slight intercellular spaces
Present in some of the bundles	D-mainly one SD-2 C-2 Protoxylem present	Absent	Present in the cortex but absent in the other zones	Absent	Absent	Present	Thin-walled round to oval parenchyma cells with slight intercellular spaces seen occasionally

bigger than the vascular part, but in my specimen the preserved portion of the cortex does not show any fibrovascular bundle. From the nature of the specimen it appears that even if the bundles are present they are not very much elongated. The characters which show some similarity between the

above three species and the specimen described in this paper are (1) the absence of the stegmata, (2) the absence of radiating and tabular parenchyma, (3) the absence of fibrous bundles, and (4) the compact nature and shape of the ground parenchyma cells. Even though the ground parenchyma cells are compact in all these four species their sizes and shapes are different in each. They are angular thick-walled cells in *P puratanam*, angular elongated cells in *P narayanai* and isodiametric rounded cells in *P niaheshwaru*. But in my specimen the ground parenchyma cells are of varying sizes and round to oval in shape. The characters of all these four species are noted in Table I.

The above-mentioned points incorporated in Table I show that on all the important criteria this specimen differs from the other three species. It is best kept as a new species.

It is, therefore, with great pleasure that I name this species *Palmaxylon sahnii* after the late Prof. B. Sahnii who was the first to take up a serious and exhaustive study of the fossil palms found in this area.

Following the combined scheme of Von Mohl (1845) and Stenzel (1904) for the classification of the artificial genus *Palmaxylon* this specimen of *Palmaxylon sahnii* can be referred to the *Mauritia* section. In this group the outer bundles are crowded, their fibrous part being greater than vascular part. The inner bundles are far apart, their fibrous part being smaller than the vascular.

### DIAGNOSIS

*Cortex* present with fibrous bundles and compact ground tissue

*Dermal zone*—Fibrovascular bundles regularly orientated, 245–320 per cm<sup>2</sup>, mostly elongated in form; diameter 0.2–0.5 mm, *f/v* ratio 0.5/1–1.4/1; median sinus concave and auricular lobes rounded; xylem vessels usually one, sometimes two or a group of vessels together. Stegmata, fibrous bundles, radiating and tabular parenchyma absent. Ground tissue compact with thin-walled parenchyma cells.

*Sub-dermal zone*—Bundles more or less regular, 150–170 per cm<sup>2</sup>; *f/v* ratio 0.4/1–1/1; bundles with different sizes and shapes; diameter 0.3–0.6 mm. Median sinus concave to flat and auricular lobes round. Main xylem vessels usually two, excluded sometimes a number of xylem vessels grouped together; protoxylem present; no fibrous bundles, stegmata, radiating or tabular parenchyma and posterior sclerenchyma. Leaf-trace



bundles bigger in size, fused bundles present. Ground parenchyma compact with thin-walled round to oval cells.

*Central zone*.—Irregularly orientated bundles; 80–125 per cm.<sup>2</sup>; different sizes and shapes; *f/v* ratio 0·3/1–0·8/1; diameter 0·5–0·8 mm. Median, sinus concave to flat, auricular lobes round, main xylem vessels mostly two, protoxylem present; phloem preserved. Stegmata and fibrous bundles absent. Leaf-trace bundles seen clearly. Ground parenchyma same as in the dermal and sub-dermal zones.

Locality—Mohgaon Kalan.

Age—Eocene.

Type specimen—M 15 (Kept in the Botany Department, Lucknow University).

#### SUMMARY

A new species of petrified palm wood has been described from the Deccan Intertrappean beds near Mohgaon Kalan area, Madhya Pradesh. It differs from all the other known Indian fossil palms in some important characters and has been designated *Palmoxylon sahnii*. It can be referred to the *Mauritia* section of petrified palms.

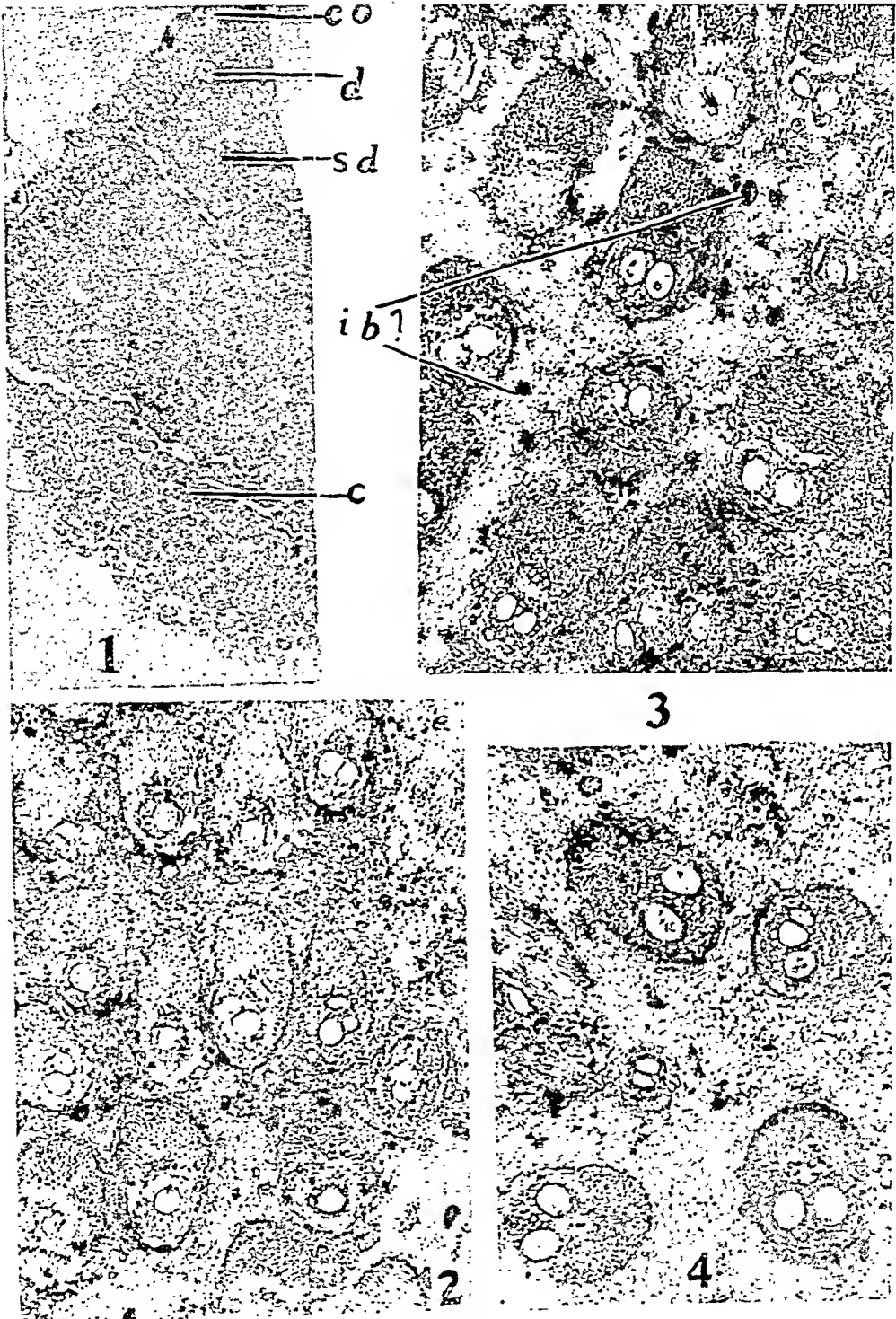
#### ACKNOWLEDGEMENTS

I am extremely grateful to Professor A. R. Rao for his kind and valuable guidance. I am also thankful to Dr. Uttam Prakash for many useful suggestions.

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FIGS. 1-4

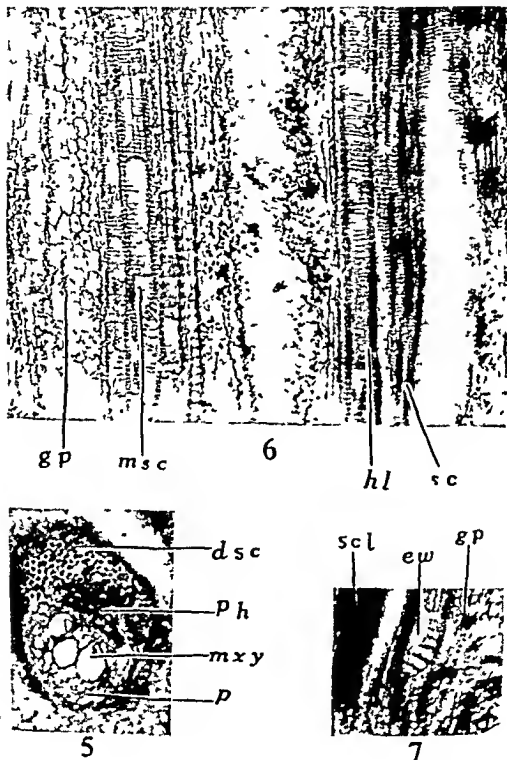


FIG. 2-

EXPLANATION OF PLATES II & III

PLATE II

- PHOTO 1. Transverse section of the specimen,  $\times 1\frac{1}{2}$ . *co*, cortical; *d*, dermal; *sd*, sub-dermal; *c*, central zone.
- PHOTO 2. Fibrovascular bundles from the dermal zone,  $\times 40\cdot 1$ .
- PHOTO 3. Fibrovascular bundles from the sub-dermal zone. *ib*, idioblast?,  $\times 41$ .
- PHOTO 4. A few fibrovascular bundles from the central zone,  $\times 38\cdot 5$ .

PLATE III

- PHOTO 5. One fibrovascular bundle from the central zone showing all the tissues. *dsc*, dorsal sclerenchyma; *mxy*, metaxylem; *p*, parenchyma; *ph*, phloem,  $\times 16\cdot 5$ .
- PHOTO 6. Longitudinal section showing the pitting of the vessels. *gp*, ground parenchyma; *hl*, helical type; *msc*, multiseriate scalariform type; *sc*, scalariform type,  $\times 138\cdot 7$ .
- PHOTO 7. Longitudinal section showing an obliquely placed end-wall. *ew*, end-wall; *gp*, ground parenchyma; *scl*, sclerenchyma,  $\times 102$ .

# ARGEMONE MEXICANA LINN.

## II. Morphological and Structural Studies in Some Floral Abnormalities

BY L D KAPOOR, F A S C AND B M SHARMA

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Received August 13, 1963

### INTRODUCTION

CONTROVERSIAL value of evidence offered by plant abnormalities has been the source of intense discussion among morphologists from the time of Linnaeus onwards. Bose<sup>1</sup> has effectively reviewed pertinent literature attaching positive importance to the value of these manifestations in the solution of problems of morphological interest. Joshi<sup>2,4</sup> reported some remarkable abnormalities in *Argemone mexicana* and discussed their possible cause and phylogenetic significance. But his conclusions are neither supported by anatomical studies nor by morphological illustrations of abnormal stamens during his latter report.

During the course of an earlier investigation the authors<sup>5</sup> conducted an extensive survey of wide population of *A. mexicana* growing in this region. In addition to abnormalities listed by Joshi (*loc cit*), it has been possible to supplement his investigations with a few more interesting observations. The latter have necessitated the submission of the following brief account.

### MATERIAL AND METHODS

Material for the present study was collected from close vicinity of Regional Research Laboratory, Jammu, and fixed and preserved in formalin-aceto-alcohol. Dehydration and paraffin embedding were carried on in the customary way. Parowax blocks were sectioned at 10-12 $\mu$  thickness and the sections were stained with safrin-fast green combination. Abnormal and normal petals and stamens were cleared in chloral hydrate-glycerine mixture (5:2) kept warm at 70°C and stained with safranin to bring out their vasculature pattern. For epidermal studies temporary mounts of petals in 10 per cent glycerine were examined.

### OBSERVATIONS

A comprehensive description of normal flowers has been furnished by Joshi.<sup>2</sup> Flowers shown in Plate IV exhibit various abnormalities which are discussed below.

*Sepals*.—Abnormal sepals observed do not deviate much from the normal structures except for their extraordinarily large size which is nearly twice that of their normal counterparts (Plate IV). The prickles are sparse and the vascular strands much strongly developed. All the sepals may be sometime, though rarely, united at the base into a circular collar-like disc.

*Petals*.—Transparencies of one normal and three abnormal petals are photographed in Plate V. Normal petals are yellow-coloured, thin and membranous with a slightly thickened base. Vascular strands diverge apart from each other immediately after their entry into the petal base. Their further ramifications and anastomoses, resulting into typical vascular network of normal petal, take place in the way exhibited by Plate V, Fig. 1.

A transection of normal petal is figured in Text-Fig. 1 (A). Detailed structure of the areas delimited by the large and small rectangle in Fig. A is shown in Figs. G and M respectively. Rounded, oval or irregular parenchymatous mesophyll cells with large air-spaces are bounded by upper and lower epidermal layers of the wing [Text-Fig. 1 (A)]. Normal epidermal cells are narrow,  $\pm$  rectangular, with sinuous walls and a few stomata [Text-Fig. 2 (a, a')].

Morphology and anatomy of the abnormal petals is described as under. The first abnormal stage (Plate II, Fig. 2) is characterized by the following morphological features:—

(i) Pale green or green colour, (ii) spatulate outline, (iii) relative increase in thickness, (iv) a lateral coming together of chief vascular strands after their entry into the petal base and finally (v) elaboration of the vascular frame into an atypical network which tends to approach more towards that of the leaf than of petal. A transection of the aberrancy under reference is shown in Text-Fig. 1 (B). Histological structure of midrib and wing, marked by straight lines in Text-Fig. 1 (B), is shown in detail in Text-Fig. 1 (H and N) respectively. Salient anatomical features of this stage are: (i) wider and better developed xylem vessels, (ii) feebly differentiated phloem fibres, (iii) appearance of loose chlorenchyma with inter-cellular spaces and (iv) the larger size of parenchymatous cells. In the wing the mesophyll gets differentiated into a few layers thick palisade tissue and several cells thick spongy parenchyma, the latter being composed of rounded or oval thin-walled cells with large air-spaces [Text-Fig. 1 (N)]. The palisade consists of slightly elongate chlorenchymatous cells with numerous interstices. Epidermal cells of this stage are characterized by an increase in the number of stomata and a straight straightening of cell-walls, accompanied by the loss of their sinuous nature [Text-Fig. 2 (b, b')].

The next abnormal stage of petal is interesting inasmuch as it combines the following distinctive features (i) development of a distinct midrib-like region, (ii) parallel course of chief vascular supplies up to the middle of petal (iii) a still further elaboration of vasculature, and (iv) appearance of lateral dorsal and ventral prickles (Plate V, Fig. B)

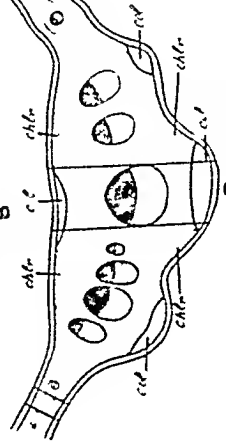
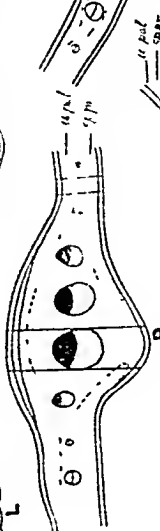
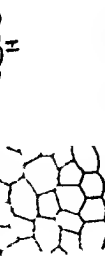
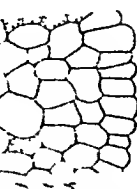
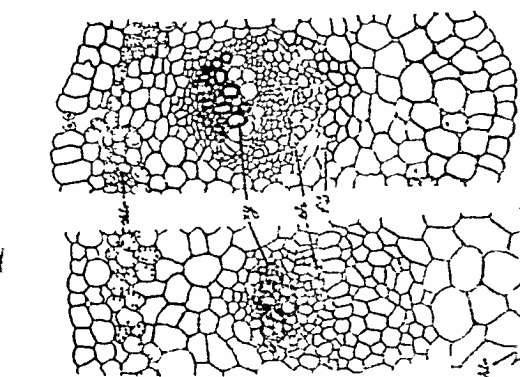
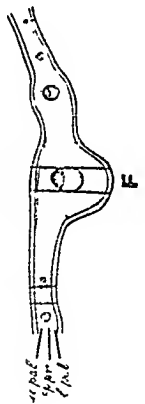
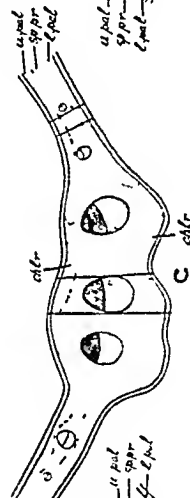
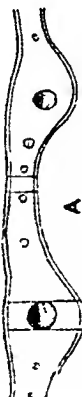
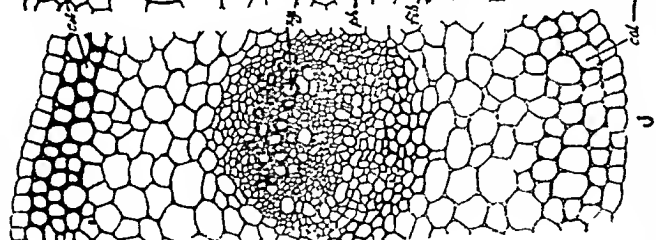
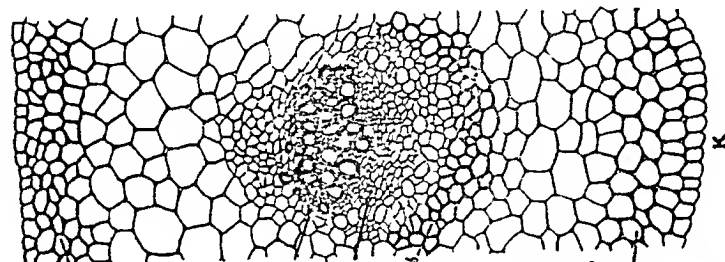
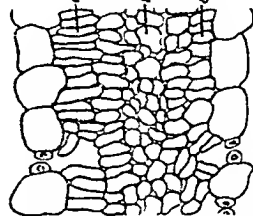
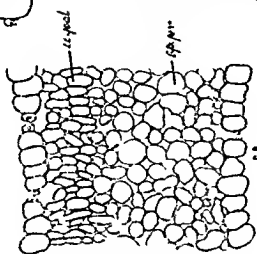
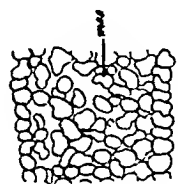
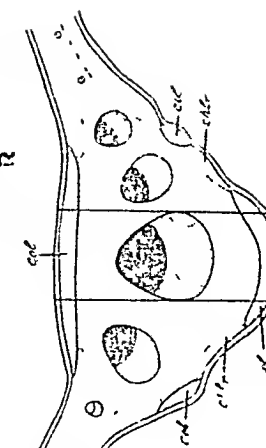
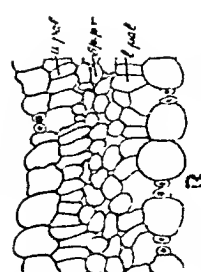
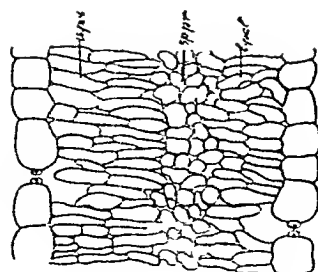
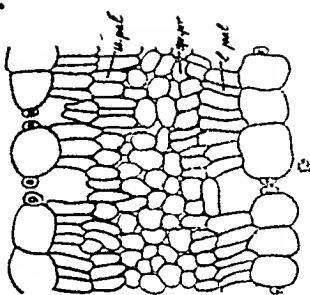
A transection of abnormal petal at this stage is delineated in Text Fig. 1 (C). Histological details of midrib and wing are illustrated with Text Fig. 1 (I and O) respectively. Chief anatomical interest at this stage lies in (i) slightly thickened phloem fibres (ii) 2-8 layers of thickened parenchyma immediately beneath the lower epidermis and (iii) appearance of chlorenchymatous bands [Text Fig. 1 (C)] in the parenchymatous region immediately below the upper and lower epidermis. Spongy parenchyma in the wing is bounded by the upper and lower palisade layers. Laterally apposed columnar cells of upper palisade more or less closely approach those of the leaf, whereas cells of lower palisade are only slightly modified chlorenchymatous cells [Text-Fig. 1 (O)]. Epidermal peels of this petal are characterized by abundance of stomata and shorter and more or less polygonal cells [Text-Fig. 2 (c, c')]

Highest degree of abnormality is attained at the third stage, shown in Plate V (Fig. 4), where the general appearance and shape gets completely altered. Were it not for its topographical situation, this stage could easily have been overlooked for ordinary foliage. The petal margin becomes wavy, lacerated and prickly and approaches closely, though not entirely, to that of the leaf. Vascular frame also approximates closely to that of the leaf.

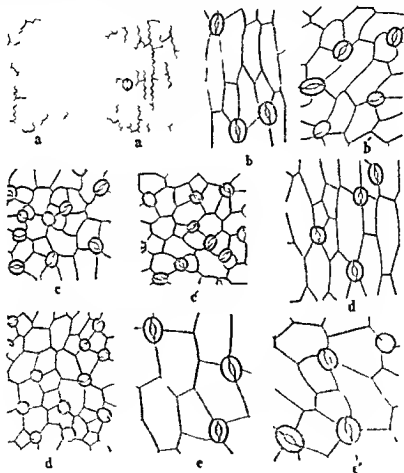
A transection of the aberrancy at this stage is figured in Text Fig. 1 (D). General plan of the tissue distribution seems to be in agreement with that of the leaf [Text Fig. 1 (E)]. Midrib regions demarcated by straight lines in Text Fig. 1 (D and E) are detailed in Text-Fig. 1 (J and K) respectively. Chief anatomical details of this stage in common with the histology of normal leaf are (i) general outline of transection (ii) comparatively stronger and thicker vascular bundles (iii) presence of a strong cap of phloem fibres and (iv) 2-3 layers of collenchymatous cells beneath the upper and lower epidermis each. The collenchyma alternates with bands of chlorenchyma beneath the lower epidermis [Text Fig. 1 (D and E)]. Transections of wing marked in Figs. 1 (D and E) by smaller rectangles are shown in detail in Figs. 1 (P and Q) respectively. Epidermal peels of the petal under reference are shown in Text Fig. 2 (d, d') while those of a normal leaf are illustrated in Text Fig. 2 (e, e').

*Stamens*—Stamens of the normal flowers possess slender filaments with a weak staminal strand. The anthers are tetralocular and longitudinal.





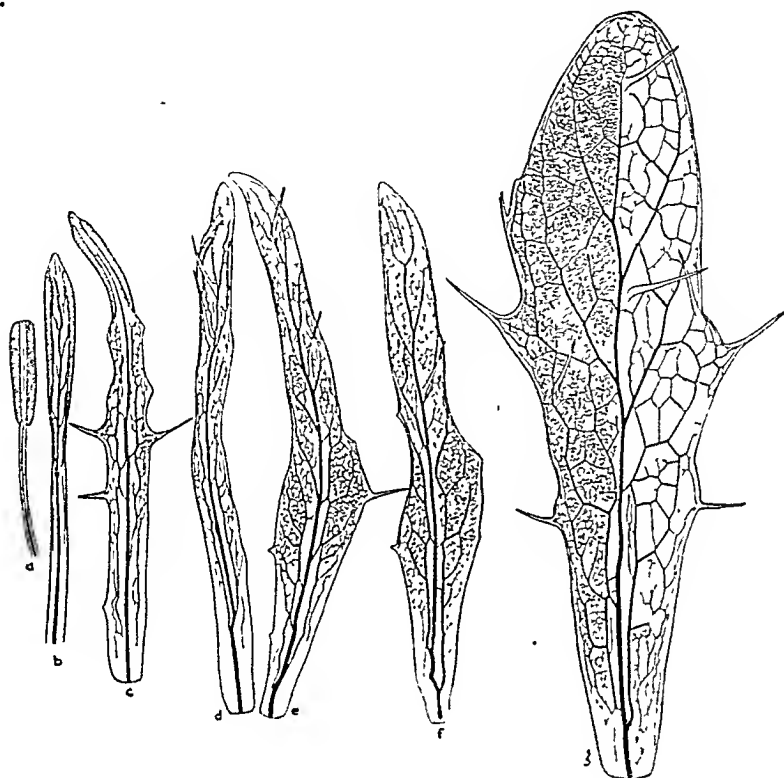
dehiscing [Text-Fig 3 (a)] Abnormal stamens arranged in order of increasing degree of deviation from normal stamen are also sketched in Text Fig 3 (b-g) The stamen shown in Text Fig 3 (g), though completely leaf like, does not approach *A. mexicana* leaf specifically Unlike the leaf, it receives a single staminal strand at its base which, as a result of its further branching and finer ramifications of subsequent branchlets, accounts for the vasculature of stamen A transection of this stamen is shown in Text Fig 1 (F) Text Fig 1 (L) illustrates histological details of the region passing through midrib



TEXT FIG. 2

Furthairn and Kapoor,<sup>2</sup> and subsequently Kapoor and Sharma (*loc cit*) reported total absence of laticiferous tubes in stamens Strikingly enough the vascular supply of abnormal stamen at this stage has been observed to be accompanied by laticifers Detailed structure of the wing in transection corresponding to the portion demarcated by straight lines in Text Fig 1

(F), is shown in Text-Fig. 1 (R). Anatomical structure of stamens *e* and *f* (Text-Fig. 3) is similar to that of *g*, while the structure of stamens *b*, *c* and *d* is precisely similar to that of the stages described by Joshi in his earlier communication.



TEXT-FIG. 3

### DISCUSSION AND CONCLUSIONS

Large and showy yellow-coloured flowers of *A. mexicana* constitute the chief allure for a casual observer, with the result that frequently occurring green aberrant forms may generally remain unnoticed in green foliage. Both the normal as well as abnormal flowers are met with on the same plant. Initiation of the abnormal flowers takes place late in the season. The authors have been able to discover several (nineteen) plants bearing abnormal flowers from three distantly situated localities in Jammu.

Whatever the cause or phylogenetic significance of these deviations, they are interesting inasmuch as they suggest extreme degrees of phyllody. All gradations of abnormality for a particular floral whorl (gynoecium excepted) may be met with in the same flower or distributed in different flowers of the

same individual or different individuals. Were these aberrancies of a particular organ arranged in ascending sequence in accordance with increasing extent of deviation from the normal a series could easily be constructed (Plate V and Text-Fig. 3). The series read in the reverse direction would tentatively explain the origin of each of such organs from a foliar ancestor lending support to the hypothesis of 'flower as a condensed vegetative shoot'.

Histological structure of these forms (Text Figs. 1-2) may bear a further testimony to their external manifestations. Irrespective of any other importance, they may be regarded significant so far as they provide with a working plan for the derivation of the presently discussed organs from the ordinary foliage leaf.

### SUMMARY

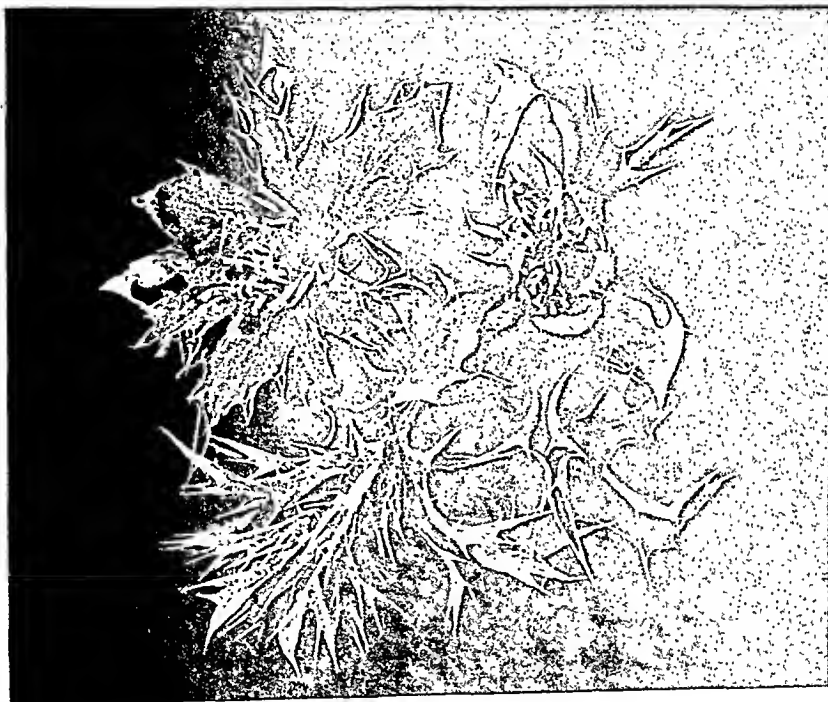
Some floral abnormalities, for *Argemone mexicana* have been studied morphologically and anatomically. Aberrations have been regarded important in deriving the foliar origin of the organs discussed in the present report. Vascular supply of extremely abnormal stamens has been found to be accompanied by laticifers.

### ACKNOWLEDGEMENTS

The authors owe a deep debt of gratitude to Dr. I. C. Chopra for his interest and encouragement, to Dr. A. C. Joshi for supplying them with relevant literature and to Mr. B. K. Abrol for his ample help during this study. They are also thankful to Mr. S. Sarup for his generous assistance with photography.

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Showing two abnormal flowers.

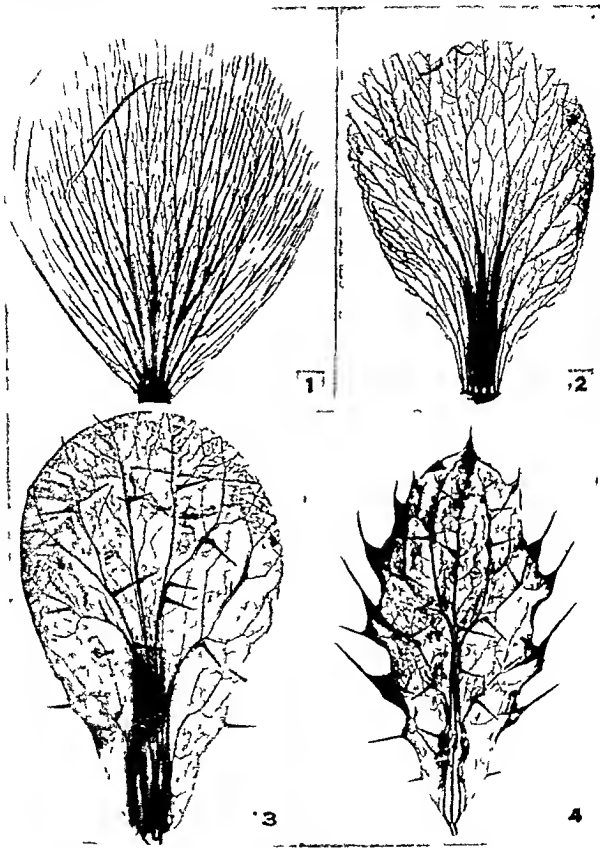


FIG. 1 Normal petal.

FIGS. 1-4

FIG. 2. Abnormal petal appearing as normal in gross appearance.

## EXPLANATION OF TEXT-FIGURES

TEXT-FIG. 1. Fig. A. T.S. normal petal. Figs. B-D. Transections of abnormal petals shown in Plate IV (Figs. 2-4). Fig. E. T.S. foliage leaf. Fig. F. T.S. abnormal stamen shown in Text-Fig. 3 (*g*). Fig. G. Histological details of normal petal midrib in T.S. Figs. H-J. Histological details of midrib region of abnormal petals corresponding to Figs. B-D respectively. Fig. K. Histological details of leaf midrib in T.S. Figs. M-R. Detailed structure of the wing region in T.S., corresponding to the areas delimited by short rectangles in Figs. A-F respectively. (Figs. A-F,  $\times 88$ ; Figs. G-L,  $\times 350$ ; Figs. M-R,  $\times 520$ .)

TEXT-FIG. 2. Figs. *a*, *b*, *c* and *d*, upper epidermal cells of respective petals shown in Plate V (Figs. 1-4). Figs. *a'*, *b'*, *c'* and *d'* Corresponding lower epidermal cells of the same stages. Figs. *e* and *e'*. Upper and lower epidermal cells of leaf. (Figs. *aa'* *ee'*.,  $\times 875$ .)

TEXT-FIG. 3. Fig. *a*. Normal stamen. Figs. *b-g*. Abnormal stamens in ascending sequence of abnormality. (Figs. *a-g*.  $\times 14$ .)

(*chl*., chlorenchyma; *col*., collenchyma; *fib*., fibres; *lat*., laticifers; *l. pal*., lower palisade; *ph*., phloem; *sp.pr*., spongy parenchyma; *u.pal*., upper palisade; *xy*., xylem.)

# SEX-EXPRESSION IN SOME NON-COMMERCIAL CITRUS VARIETIES

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(Horticultural Research Institute, Saharanpur\*)

Received August 13, 1963

(Communicated by Dr L D Kapoor F.A.Sc.)

FAILURE to set fruits in some of the citrus varieties has greatly handicapped their extensive plantation (Singh, *et al*, 1957) Self-incompatibility has been suggested as one of the probable causes for this trouble (Singh and Dhuria, 1960) However, it was felt that the ratio of hermaphrodite and staminate flowers may greatly affect the fruit setting, since the citrus trees bear hermaphrodite and staminate flowers simultaneously Although some information is available for the commercial varieties, however, such data is lacking in the case of non-commercial varieties, which offer a potential hybridization plant material source With this view these studies were carried out in seventeen varieties growing at the Horticultural Research Institute, Saharanpur The varieties studied were —

1	Sadaphal	<i>C semperflorens</i> Lush
2	Dominica	<i>C limon</i> Burm
3	Lemon Eureka	Do
4	Lemon Seedless	Do
5	Lemon Kaghzi	Do
6	Lemon Oval	Do
7	Jambheri	<i>C Jambheri</i> Lush
8	Jambheri Brown	Do
9	Florida Rough	Do
10	Italian 76	Do
11	Amilbed (Gajanimma)	<i>C pennivesiculata</i> Tanaka
12	Sour orange	<i>C aurantium</i> Linn

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\* Present address Regional Research Laboratory Jammu (Tawi)



- |                  |   |
|------------------|---|
| 13. Rangpur lime | .. <i>C. limonia</i> Osbeck.                  |
| 14. Karna Khatta | .. <i>C. karna</i> Raf.                       |
| 15. Sweet lime   | .. <i>C. limettioides</i> Tanaka.             |
| 16. Kaghzi lime  | .. <i>C. aurantifolia</i> Swingle.            |
| 17. Sour Galgal  | .. <i>C. galgal</i> Sp. Nov. Motial and Singh |

### RESULTS AND DISCUSSION

All the varieties were observed to bear hermaphrodite as well as staminate flowers. The percentage of staminate flowers varied greatly according to the variety and season.

(a) *Percentage of staminate and hermaphrodite flowers.*—The percentage of staminate and hermaphrodite flowers was calculated from the number of staminate and hermaphrodite flowers recorded separately for seven days. The quantitative averages are summarized in Table I.

A perusal of the Table shows that the highest percentage of staminate flowers was found in Sweet lime during 1961, while the highest percentage of hermaphrodite flowers was observed in lemon Kaghzi.

The percentage of Staminate flowers was greatly influenced by the season of flowering. In lemon Eureka and Lemon Seedless, daily counts of staminate and hermaphrodite flowers, for seven days were made in July, December and March during the flowering seasons. The data presented in Table I shows that the minimum percentage of staminate flowers was produced in the December flowering, whereas the spring bloom recorded the highest percentage.

A close comparison of these results with the meteorological data given in Table II indicates a correlationship between the percentage of hermaphrodite flowers and temperature on the one hand and relative humidity on the other. Thus, the low temperatures and relatively high humidities during December produced a higher percentage of hermaphrodite flowers as compared to the relatively high temperatures and low relative humidity during March.

With the advancement of the season, a rise or fall in the temperature also affected the percentage of staminate flowers opening in that period. When the temperatures were lower, the number of hermaphrodite flowers opening was greater. Similar conclusions have been drawn by various other workers also (Sauer, 1954; Singh and Dhuria, 1960).

TABLE I

*Staminate and hermaphrodite flowers in different varieties of citrus*

Variety	Total flowers observed	Stami- nate flowers	Herma- phrodite flowers	% of staminate flowers	% of herma- phrodite flowers
<i>Spring Bloom, 1961</i>					
Jambheri	378	320	58	84.65	15.35
Jambheri Brown	453	405	48	89.40	10.60
Florida Rough	305	286	19	93.77	6.23
Italian 76	616	425	191	68.99	31.01
Sweet lime	1516	1445	71	95.31	4.69
Sadaphal	318	195	123	61.32	38.68
Sour orange ..	1217	562	655	46.17	53.83
Rangpur lime ..	854	421	433	49.29	50.71
Dominica ..	221	119	102	53.84	46.16
<i>Rainy season Bloom, 1961</i>					
Lemon Eureka ..	315	218	97	69.84	30.16
Lemon Seedless ..	170	123	47	78.23	21.77
<i>December Bloom 1961</i>					
Lemon Eureka ..	55	31	24	56.36	43.64
Lemon Seedless ..	62	36	26	58.06	41.94
<i>Spring Bloom, 1962</i>					
Jambheri .. ..	809	538	271	66.50	33.50
Jambheri Brown ..	1091	561	530	51.51	48.49
Florida Rough ..	999	570	429	57.05	42.95
Italian 76 .. ..	653	185	468	28.33	71.67
Sweet lime .. ..	750	675	75	90.00	10.00
Sour Galgal ..	939	902	87	91.20	8.80
Amilbed (Gajanimma)	628	546	82	86.53	13.47
Lemoo Kaghzi ..	471	24	447	5.09	94.91
Lemon Oval ..	461	253	208	54.88	45.12
Kaghzi lime ..	930	797	133	85.69	14.31
Karna Khatta ..	414	229	185	55.31	44.69
Lemon Eureka ..	604	525	79	86.92	13.08
Lemon Seedless ..	531	475	56	89.45	10.55

All the averages are from seven days records.

TABLE II  
*Meteorological records*

Month	Temperature		Humidity % at hours	
	Maximum ° C.	Minimum ° C.	0720	1420
1960				
December .. ..	23.7	5.5	87	32
1961				
January .. ..	20.5	7.0	92	49
February .. ..	22.1	8.0	81	32
March .. ..	30.1	12.6	71	25
April .. ..	36.4	15.6	50	19
July .. ..	36.6	26.4	87	70
August .. ..	31.4	24.7	90	75
September .. ..	33.4	24.2	85	63
December .. ..	19.3	4.0	53	53
1962				
January .. ..	19.3	4.8	90	45
February .. ..	22.7	9.5	88	47
March .. ..	27.3	11.9	72	31
April .. ..	35.2	17.8	53	20

(b) *Percentage of staminate flowers opening at different hours of the day.*—Records for 13 varieties were maintained for seven days for the number of staminate flowers opened at hourly intervals from 6 a.m. to 6 p.m. The means are presented in Table III.

Table III reveals that the highest percentage of staminate flowers opened either in the early or late hours of the day. Figure 1 shows that at 6 a.m. and 7 a.m. a higher number of staminate flowers opened but at 8 a.m. this

TABLE III

Percentage of staminate flowers in different citrus varieties at different hours of the day

Variety	Percentage of staminate flowers opening at hours													
	6	7	8	9	10	11	12	13	14	15	16	17	18	
Karna Khasa	78.0	80.0	58.8	60.0	60.8	59.5	42.8	45.4	52.4	53.6	52.0	50.3	72.0	
Sweetlime	96.4	92.6	81.8	86.9	85.7	96.6	84.0	89.5	93.0	93.6	95.0	96.7	100.0	
Italian 75	50.0	35.7	22.2	20.6	22.8	16.5	29.7	27.1	34.1	23.8	20.7	42.6	45.7	
Lemon Oval	86.5	83.3	33.3	58.6	41.6	54.0	50.0	41.9	50.0	70.6	54.8	67.1	81.8	
Kagadi lime	100.0	89.7	85.7	89.8	83.2	79.7	82.9	82.7	89.3	85.0	90.0	67.2	67.1	
Pililla kough	73.7	68.8	52.1	62.7	51.4	50.6	60.7	58.7	57.2	58.3	50.8	55.3	67.5	
Janberi	70.0	74.1	66.6	74.4	62.1	56.2	68.9	61.0	63.6	63.4	61.2	63.4	80.0	
Jamkei Brown	58.1	40.0	39.4	56.1	53.8	46.1	50.4	51.5	56.2	48.6	52.7	54.0	65.4	
Amilbed (C. adina)	87.5	86.7	73.9	87.9	80.3	84.7	83.0	84.4	87.0	100.0	94.4	100.0	100.0	
Amilbed	97.8	95.0	88.1	88.4	89.3	82.7	88.6	89.6	90.7	94.3	100.0	100.0	100.0	
Lemon Furka	90.9	75.0	65.0	89.2	80.4	84.1	88.5	85.1	90.7	97.3	90.0	97.8	96.8	
Lemon Seelias	100.0	100.0	94.3	94.7	84.6	86.1	91.3	87.9	92.4	91.7	91.4	91.7	96.0	
Lemon Nagdi	11.1	7.7	0.0	10.5	8.5	1.2	0.0	2.6	2.7	5.3	7.8	6.0	14.8	

\* N.B.—All the averages are from the data recorded for seven days

percentage decreased and again rose at 9 a.m. Further increase or decrease in percentage of staminate flowers depended upon the individual variety with a definite increase in staminate flowers towards the end of the day. In most of the varieties, the optimum percentage of hermaphrodite flowers opened between 10 a.m. and 1 p.m.

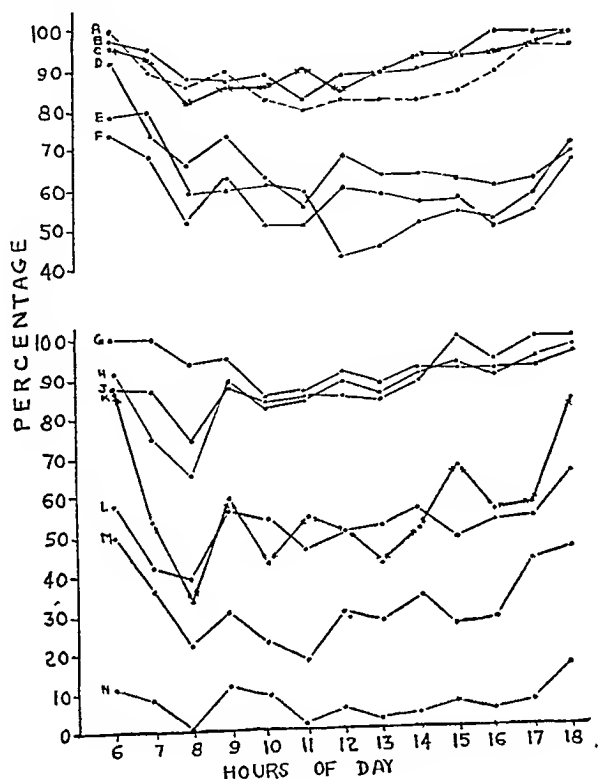


FIG. 1. Percentage of staminate flowers appearing at different hours of day.

A. Kaghzi lime; B. Sour Galgal; C. Sweet lime; D. Jambheri; E. Kama Khatta; F. Florida Rough; G. Lemon Seedless; H. Lemon Eureka; J. Amilled (*Gajanimma*); K. Lemon Oval; L. Jambheri Brown; M. Italian 76; N. Lemon Kaghzi.

### SUMMARY

1. The highest percentage of staminate flowers was recorded in Sweet lime, and the least in Lemon Kaghzi, amongst the seventeen non-commercial citrus varieties studied at the Horticultural Research Institute, Saharanpur.

2. The maximum number of staminate flowers were found to open either in the early or late hours of the day.

## ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Dr. L. B. Singh for his valuable guidance during the course of these studies.

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# STUDIES ON THE SPECIFICITY OF HAEMOLYMPH FROM DIFFERENT INSECTS FOR THE CULTURE OF TISSUES FROM OTHER INSECTS

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(Communicated by Dr. S. Krishnaswamy, F.A.Sc.)

## INTRODUCTION

UP TO this time in almost all the cases of insect tissue culture (comp. reviews by Murray and Kopech, 1953; Loeb, 1957; Hartzell, 1958; Day and Grace, 1959) haemolymph or tissue extract has been used from the same insect from which the tissue was taken. This has been done perhaps from the idea that the particular haemolymph might contain all the necessary requirements for the particular tissue. Papers coming out on the biochemistry of haemolymph, (comp. reviews by Buck, 1953, Wyatt, 1961), however, do not endorse this view. No doubt, there are some differences from species to species, specially in the ratios of organic and inorganic components, some antigenic structures; yet the general fundamental similarities like high potassium and magnesium and low sodium content, the high level of free amino-acids and their frequent contribution to cations rather than anions, the presence of high concentrations of glutamine, proline and one or more of the basic amino-acids, arginine, lysine and histidine are important. The other common features are the presence of citrate as organic acid, high content of phosphates and the occurrence of the non-reducing disaccharide trehalose, as the main blood sugar.

An experiment was set to examine the specificity of haemolymph from different insects by trying various combinations of blood and tissue. This was thought important as many of the insects could supply a greater quantity of haemolymph often due to their bigger sizes whereas from others blood which did not show tyrosinase activity, could be obtained which could be exploited for the culture of tissues from other insects too.

## MATERIALS AND METHOD

The specimens used for the experiment were the larvae of three species of insects with different food habits, viz., *Galleria mellonella* L., *Antheraea*

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*pernyi*, Guér and *Melolontha melolontha* L. *Galleria mellonella* were reared in the laboratory on Haydak's artificial medium (Balazs, 1958) *Antheraea pernyi* were reared in the laboratory on oak leaves and *Melolontha melolontha* in sawdust on potatoes. For the experiment tissues from the late last instar larvae of all insects were used. Pieces of intestinal tissue from *G. mellonella* were put in the medium (modified Trager's C<sub>4</sub>, Sen Gupta, 1961) with haemolymph from *G. mellonella*, *A. pernyi* and *M. melolontha*. Similarly, intestinal tissue from *A. pernyi* were put in the medium with haemolymph from *A. pernyi*, *G. mellonella* and *M. melolontha*, and intestinal tissue from *M. melolontha* were put in the medium with haemolymph from *M. melolontha*, *G. mellonella* and *A. pernyi*. With each combination five replications were taken and average of them noted. All the tissues were cultured following standard roller-tube technique.

### RESULTS

Survival and growth of intestinal tissue explants from *G. mellonella*, put in the modified Trager's C<sub>4</sub> with different haemolymphs when interpreted in terms of average survival period, average growth period and the percentage

TABLE I

Table showing the survival period, the growth period and the percentage of tissues from different insects growing in basic modified Trager's medium C<sub>4</sub> with different haemolymphs

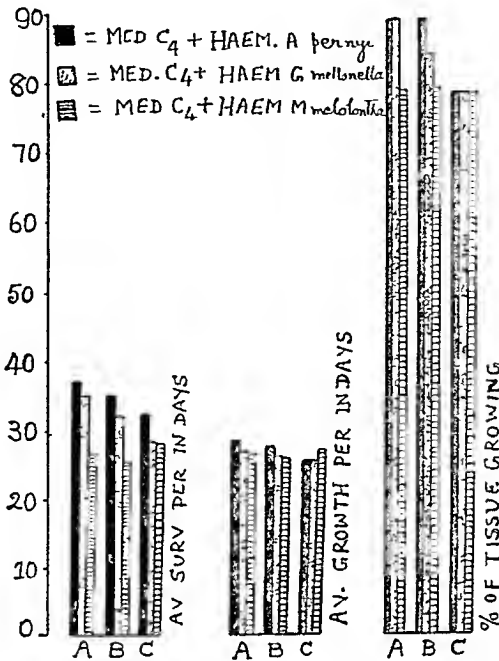
Tissue from	MEDIA								
	C <sub>4</sub> + h.A.p			C <sub>4</sub> + h.G.m			C <sub>4</sub> + h.M.m		
	S	G		S	G		S	G	
	sp	ep	pg	sp	ep	pg	sp	ep	pg
<i>Galleria mellonella</i>	37	28	90%	35	27	90%	26	25	80%
<i>Antheraea pernyi</i>	35	28	90%	32	26	85%	29	26	80%
<i>Melolontha melolontha</i>	32	25	80%	28	25	80%	28	27	80%

S, survival, G growth, sp, average survival period in days, ep, average growth period in days, pg, percentage of tissue growing, h.A.p, haemolymph, *A. pernyi*, h.G.m. = haemolymph, *G. mellonella*, h.M.m., haemolymph, *M. melolontha*

of tissue growing showed the maximum growth and survival period of the tissue with haemolymph from *A. pernyi*, followed by the tissues put in the medium with haemolymph from *G. mellonella*. As far as the percentage of tissue growing was concerned both of them appeared to be similarly effective



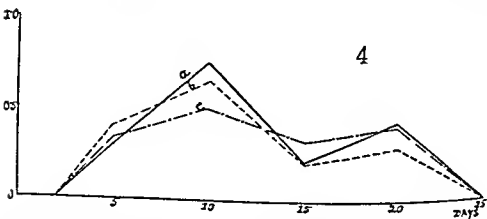
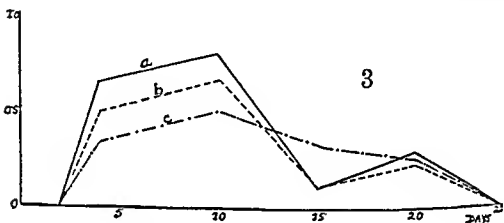
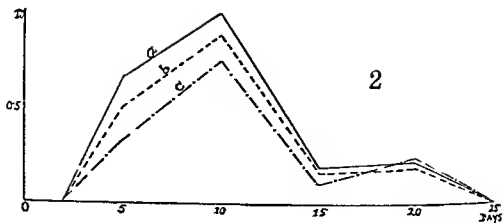
The medium with haemolymph from *M. melolontha* appeared to be the inferior in all respects.



GRAPH 1. Graph showing the comparative merits of the different combinations of the medium modified Trager's  $C_4$  with different haemolymphs for the survival and growth of intestinal tissue: A. From *G. mellonella*, B. From *A. pernyi*, C. From *M. melolontha*.

Survival and growth of intestinal tissue explants from *A. pernyi* when interpreted in terms of average survival period, average growth period and the percentage of tissue growing showed the maximum survival and growth period and the percentage of tissue growing in the medium with haemolymph from *A. pernyi*. The next best in all respects were the cultures set up with haemolymph from *G. mellonella*. In the same way survival and growth of intestinal tissue explants from *M. melolontha* when interpreted showed the maximum survival period in the medium with haemolymph from *A. pernyi*. With haemolymph from *G. mellonella* and *M. melolontha* survival periods were the same. Growth period was, however, maximum in the cultures set up with haemolymph from *M. melolontha*. In the cultures with haemolymph from *A. pernyi* and *G. mellonella* the growth periods were the same. The percentage of tissue growing in all the three combinations was the same.

The survival period, growth period and the percentage of tissues growing in different media are shown in Table I and compared in Graph 1. The conditions of cultures of tissues from different insects in different combinations



GRAPHS 2-4 Graphs comparing the rates of growth in intestinal tissue explants: 2 From *A. pernyi*, 3 From *G. mellonella*, 4 From *M. melolontha* in the medium modified Trager's  $C_4$  + 3 different haemolymphs

- a = in medium  $C_4$  + haemolymph from *A. pernyi*,  
 b = in medium  $C_4$  + haemolymph from *G. mellonella*,  
 c = in medium  $C_4$  + haemolymph from *M. melolontha*

of the modified Trager's  $C_4$  medium after 7 days of cultivation are shown in Figs. 1 to 9.

The rates of growth followed in the intestinal tissue explants from *G. mellonella*, *A. pernyi* and *M. melolontha* set up with the different combinations of the modified Trager's  $C_4$  medium in a criss-cross way showed (Graphs 2, 3, 4) that in the first phase up to the tenth day in all cases except with the tissue from *M. melolontha* the coefficients of growth had higher value in the medium with haemolymph from *A. pernyi*. In the later phases, however, around the twentieth day all the media showed lower coefficients of growth with not much of difference between them though in two sets with haemolymph from *A. pernyi* growth coefficients were slightly higher.

### DISCUSSION

Review of the above observations shows that tissues set up with haemolymph from *A. pernyi* showed the best growth. Next best were the cultures with haemolymph from *G. mellonella*. Growth in cultures set up with haemolymph from *M. melolontha* even with the tissues from *M. melolontha* was not better than the *M. melolontha* tissue with haemolymph from *A. pernyi*, which shows that there is no specificity in the action of haemolymph. There is difference in the action of haemolymph, but that appears not to be related to the species from which haemolymph has been added and the species from which tissue has been taken but on some other factor or factors. It is an admitted fact that haemolymph in some of the insects is richer in organic and inorganic components and in its suitability for the culture of different tissues that might be the more important factor than the narrow limit of species differentiation and as has been found from the above experiment in some cases haemolymph from other insect might be more suitable for the culture of tissues than the haemolymph from the same species.

### SUMMARY

Cultures were set with tissues from three species of insects, viz., *G. mellonella*, *A. pernyi* and *M. melolontha* in the basic medium (modified Trager's  $C_4$ ) with haemolymph from each of them in a criss-cross way. The results when interpreted to see if there was any specificity in the action of haemolymph for the culture of tissues from a particular insect *in vitro* showed that:

- (1) There is no specificity in the action of haemolymph.
- (2) Slight differences in the action of haemolymph for the culture of different tissues exist but that does not appear to be specific, as tissues from

all the insects showed a little better growth in the medium with haemolymph from *A. pernyi*.

(3) The differences are explained to be due to possible differences amongst different haemolymphs in the richness of some organic and inorganic components.

### ACKNOWLEDGEMENTS

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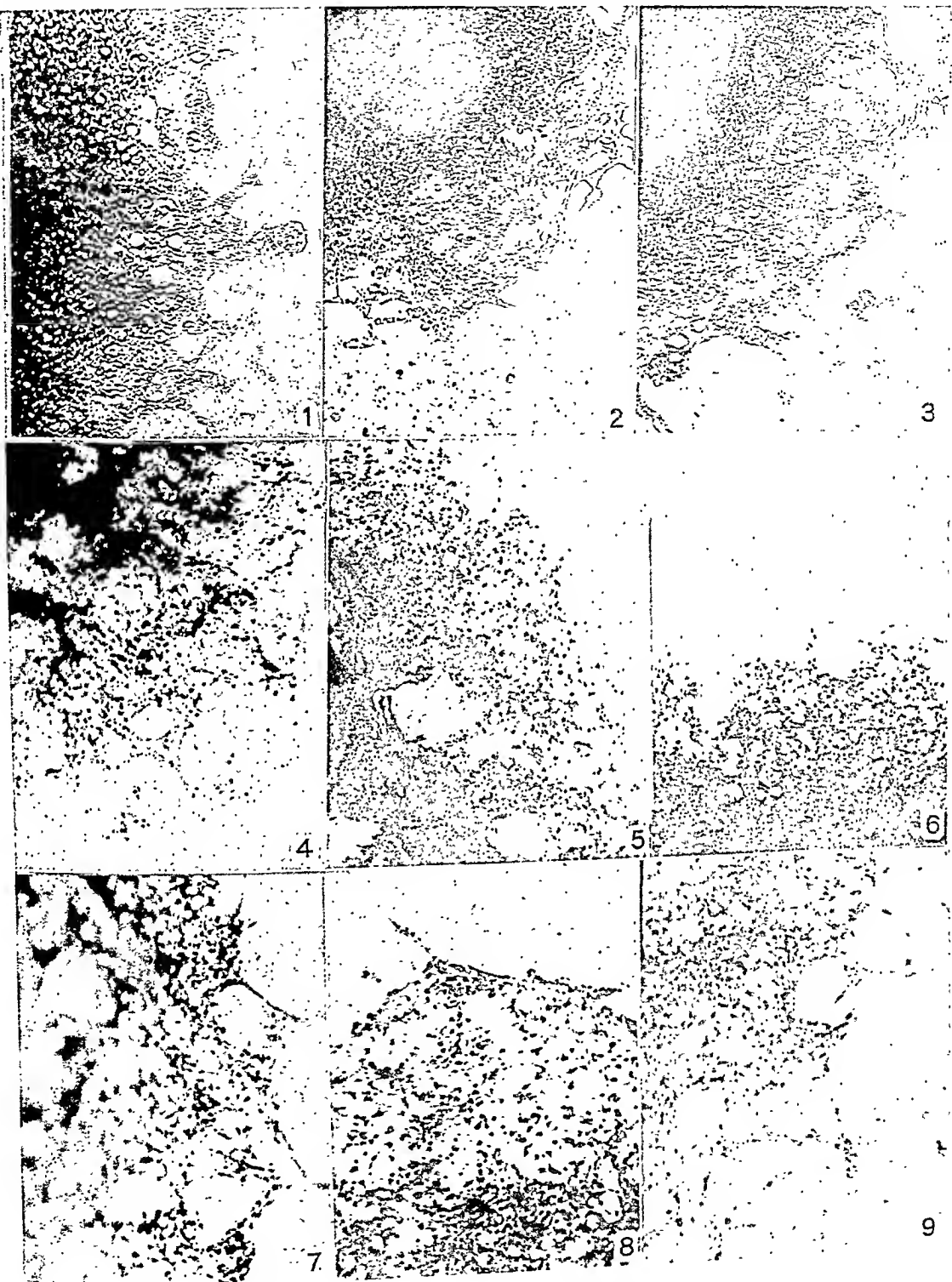
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### EXPLANATION OF PLATE VI

FIGS. 1-3. Intestinal tissue explants from *G. mellonella* after 7 days of cultivation *in vitro*.

Fig. 1. Put in the modified Trager's  $C_4$  medium with haemolymph from *A. pernyi*.

Fig. 2. Put in the medium modified Trager's  $C_4$  with haemolymph from *G. mellonella*.



FIGS. 1-9

Fig. 3. Put in the medium modified Trager's  $C_4$  with haemolymph from *M. melolontha* (all *in vitro*,  $\times 53$ ).

FIGS. 4-6. *Intestinal tissue explants from A. pernyi after 7 days of cultivation in vitro*

Fig. 4. Put in the modified Trager's  $C_4$  medium with haemolymph from *A. pernyi*.

Fig. 5. Put in the medium modified Trager's  $C_4$  with haemolymph from *G. mellonella*.

Fig. 6. Put in the medium modified Trager's  $C_4$  with haemolymph from *M. melolontha* (all stained with Giemsa,  $\times 53$ ).

FIGS. 7-9. *Intestinal tissue explants from M. melolontha after 7 days of cultivation in vitro.*

Fig. 7. Put in the modified Trager's  $C_4$  medium with haemolymph from *A. pernyi*.

Fig. 8. Put in the medium modified Trager's  $C_4$  with haemolymph from *G. mellonella*.

Fig. 9. Put in the medium modified Trager's  $C_4$  with haemolymph from *M. melolontha* (all stained with Giemsa,  $\times 53$ ).

# CONTRIBUTIONS TO A BIOCHEMICAL STUDY OF SUGARCANE

## I Potential Nitrate and Nitrate Reductase in Sugarcane and Their Possible Relation to Yield Capacity\*

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(Communicated by Prof. T. R. Seshadri, F.R.S., F.A.S.C.)

ALTHOUGH the ability of intact plant tissues to reduce nitrate has been established for many years,<sup>2, 17, 21</sup> it is only recently that the mechanism of this reaction, and the nature of the concerned enzyme, nitrate reductase, and its relation to nitrate reduction, photosynthesis and respiration in plants, have been elucidated.<sup>9, 13</sup>

In sugarcane, a detailed study of nitrate reductase has not so far been made. Preliminary tests by the present authors showed the presence of nitrate reductase activity (NRA) in the leaves, roots and stem of sugarcane. In the stem it was found to be present in a considerable amount. This is of interest since the reduction of nitrate is reported mainly in the roots<sup>3, 4, 10, 11, 18-20</sup> and shoot system<sup>5, 6, 12-14</sup> (leaves, leaf buds and meristematic portions of green tender stems) of plants. Since nitrate reduction in the plant parts, other than leaves, is coupled with respiration, and since in sugarcane stem the presence of large amount of sucrose could give rise to a free supply of glucose and fructose for respiratory needs, the NRA therein is considered to be of significance. In the present work, a study has been made of the NRA, and the nitrate N, total N and sucrose contents in sugarcane stem at different stages of growth. From a study of the relation between NRA and nitrate levels, occurring at different growth periods, the concept of potential nitrate and its possible utility in determining the superiority of a cane variety in respect of yield have been discussed.

### MATERIALS AND METHODS

The hybrid sugarcane varieties Co 475, Co 508, Co 678 and Co 453 were used for the study. The former two are earlier in ripening as compared

\* Published with the approval of the Director, Sugarcane Breeding Institute, Coimbatore 7.

to the latter two. The varieties were planted in the first week of April 1962 in five replications, each replication containing three  $20' \times 1\frac{1}{2}'$  rows per variety. The rows were spaced 3' apart. Twenty setts (three-budded) were planted per row. Ammonium sulphate at the rate of 600 lb. per acre was applied in two equal doses at 45 days and 90 days of age of the crop. Irrigation was given at weekly intervals.

Samples of stem were taken at monthly intervals from  $6\frac{1}{2}$  months of age of the crop, when there was sufficient amount of stem formation, up to 11 months of age. For sampling, each time, per variety, 2 sets of 5 main tillers, drawing two main tillers from two side-by-side growing clumps from each replication, were taken at 10 A.M. One set was used for extracting the juice for estimating NRA and true-sucrose content. The other set was used for preparing a dry sample by cutting the cane into small bits, drying rapidly at  $85-87^{\circ}\text{C}$ . in a well-ventilated electric oven, powdering and then preserving the powder. Samples of main tillers in the vegetative phase only were taken throughout.

True-sucrose content in the juice was estimated by Clerget's double polarisation method.<sup>16</sup> NRA was estimated adapting the method of Hibbard.<sup>7</sup> To 20 ml. fresh juice were added 40 ml. of 0.05 M phosphate buffer (pH 7.3), 4 ml.  $\text{KNO}_3$  solution (10 %) and 40 mg. glucose, and the pH of the contents adjusted to 7.3 with 0.2 N NaOH using phenol red as external indicator. After adding sufficient toluene, the contents were incubated at  $37^{\circ}\text{C}$ . for 20 hrs. after which they were made up to 100 ml. This solution was clarified with 12 gm. activated carbon, and 10 ml. of the clear colourless filtrate was used for nitrite estimation with  $\alpha$ -naphthylamine-sulphanilic acid reagent. The sample of activated carbon used was tested and found to be free from adsorption of nitrite.

In the powdered samples total N ( $p$ ) was determined following the A.O.A.C. procedure,<sup>1</sup> while the nitrate N( $q$ ) was estimated according to the method described by Humphries.<sup>8</sup> ( $p-q$ ) gave total N excluding nitrate N.

At  $10\frac{1}{2}$  months of age of the crop, weight of a bulk sample of 50 millable canes per variety (10 canes at random from each of the five replications) was recorded and the canes were crushed. The bulk juice sample obtained was used for recording the brix, sucrose and true-sucrose values.

In Table I are given the values for NRA, true-sucrose, nitrate N and total N (excluding nitrate N). In Table II are given the weight of 50 canes, the number and weight of canes in 15 rows, the true-sucrose per cent. in juice, and the C.C.S. (Commercial Cane-Sugar) per cent. cane, as estimated from the bulk cane sample, in respect of each variety.



## RESULTS AND DISCUSSION

From Table 1 it can be seen that a considerable amount of NRA is present in the cane stem in all the 4 varieties throughout the different stages

TABLE I

*Showing the nitrate reductase activity and the percentages of true sucrose nitrate N potential nitrate N and total N at different stages of growth*

Month of Sampling	October 1962	November 1962	December 1962	January 1963	February 1963
Age in months	6½	7½	8½	9½	10½
Variety	Nitrate Reductase Activity†				
Co 475	0 009*	0 013*	0 023*	0 018	0 015
Co 508	0 094	0 017*	0 037*	0 028	0 032
Co 678	0 160	0 021*	0 033*	0 024	0 017
Co 453	0 022*	0 027*	0 031*	0 030	0 030
True-Sucrose Per cent of Juice					
Co 475	8 3	9 7	17 1	16 5	17 5
Co 508	10 3	12 1	16 4	17 6	16 8
Co 678	7 6	9 6	12 4	14 2	15 7
Co 453	8 4	11 6	13 2	14 5	14 9
Nitrate N Per cent ‡					
Co 475	0 050*	0 064*	0 098*	0 132	0 102
Co 508	0 048	0 055*	0 075*	0 032	0 053
Co 678	0 040	0 048*	0 079*	0 081	0 073
Co 453	0 029*	0 046*	0 052*	0 071	0 046
Potential Nitrate N Per cent ‡					
Co 475	0 031	0 044	0 078	0 061	0 051*
Co 508	0 094	0 017	0 037	0 028	0 032
Co 678	0 408	0 054	0 084	0 061	0 043
Co 453	0 067	0 032	0 095	0 092	0 092
Total N (Excluding Nitrate N) Per cent ‡					
Co 475	0 33	0 28	0 24	0 28	0 21
Co 508	0 44	0 37	0 22	0 54	0 21
Co 678	0 42	0 37	0 34	0 38	0 26
Co 453	0 45	0 41	0 34	0 31	0 28

\* Values used for drawing nitrate N reductase activity graph.

† mg. nitrate N per 100 g. tissue fluid.

‡ Percentage on zero moisture basis.

of growth. But for the initial high values in Co. 508 and Co. 678, during October, the general tendency in NRA is gradually to increase up to December and thereafter to decline. It can also be noticed from the table that the true-sucrose percentage of the juices bears no relation to the NRA throughout the different stages of growth after cane formation. The relation between NRA and nitrate N levels, in the different varieties, brings out certain points of interest. In general, the gradual increase in the nitrate N values during the three months October, November and December, in all the four varieties, is followed by a gradual increase in the NRA also during these months. During January and February, however, in general, a decrease in the NRA is observed while the nitrate N tends to remain relatively at a higher level in the case of all the four varieties. The reason for this is discussed at a later stage.

TABLE II

*Data of analysis of bulk cane sample*

Variety	Wt. of 50 canes (Kg.)	No. of canes in 15 rows	Wt. of canes in 15 rows (Kg.)	True- sucrose per cent. in Juice	C.C.S. per cent. in cane	Wt. of sucrose in the canes contained in 15 rows (Kg.)
Co. 475	56.66	401	454.3	17.1	12.98	58.99
Co. 508	29.34	962	564.5	17.7	12.73	71.86
Co. 678	78.30	750	1174.5	16.1	10.78	126.70
Co. 453	45.80	840	769.5	15.7	10.90	83.87

The occurrence of sets of nitrate N and NRA values with gradual increase during October, November and December enables us to draw graphs showing the nitrate N-NRA relation for the 4 varieties. The graphs for Co. 475 and Co. 453 show that the relation between nitrate N and NRA is linear. In the case of Co. 508 and Co. 678 the graphs are drawn with the sets of values of nitrate N and NRA obtained during November and December.

The nitrate N-NRA graphs do not pass through the origin but intercept the X-axis, the reading at the point of interception being positive or negative depending on the rate of uptake of nitrate and the rate of its assimilation in the different varieties. If 'X' and 'Y' are the values of nitrate N and NRA, represented by any point on the graph, and 'a' the reading at the

point of interception, then  $(X-a)/Y$  gives the factor,  $f$ , by which the NRA is to be multiplied to get the percentage of nitrate N potentially significant (potential nitrate) in reduction.

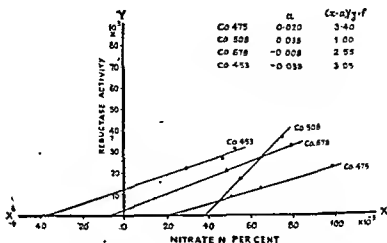


FIG. 1. Show  $n_2$  nitrate  $n$ -reductase activity relation.

When the NRA is at a very high level as observed in the varieties Co. 508 and Co. 678 during October, the assimilation of nitrate could be expected to take place at a rate far higher than its absorption thus resulting in low observed nitrate N values in disproportion to the NRA. Similarly, if NRA decreases to lower levels, and if there is a continuous uptake of nitrate, then the observed nitrate N values tend to be high in disproportion to the NRA, as seen in the case of all the four varieties during the months of January and February. Hence, under either of these two circumstances, the NRA and the observed nitrate N values, being not proportional, will be of little use in the construction of the graph showing nitrate N-NRA relation. But even in these cases it is possible to find the potential nitrate N (pN) provided the nitrate N-NRA graph is drawn, as already explained, using two or more sets of the required values and the factor,  $f$ , is calculated. In practice, it is convenient to have three or more sets of the required values by increasing the frequency of sampling.

The pN values calculated for the 4 varieties at the different stages of growth are given in Table I. pN values, being directly proportional to the NRA values, show the same trend of fluctuation. Since pN gives a measure of the quantum of nitrate reduction and consequently nitrate utilisation, its level in the plant is of interest. It can be seen from Tables I and II that in general, higher values of weight of cane as well as sugar, calculated for

15 rows at the 11th month, are accompanied by higher pN values during the 7th, 8th and 9th month of age of the plant when the grand period of growth is expected to occur in the plants. A higher level of pN during the 7th, 8th and 9th month stage of the plant can thus be considered indicative of the superiority of a cane variety in respect of yield over one with a lower level. A detailed confirmatory study of this aspect is being taken up.

On the basis of the pN values at 7th, 8th and 9th month stages, the 4 varieties may be arranged in the order of decreasing yield (calculated) as follows: Co. 678, Co. 453, Co. 475 and Co. 508. This order has been found to be so in actual experience. In the present experiment, however, Co. 475 showed the minimum weight of cane and sugar in 15 rows. This may be explainable as due to an unusually poor germination of 37% in Co. 475 as against the 55% to 62% germination observed in the other three varieties which appears to have resulted in the discrepancy in the position of Co. 475.

The total N (excluding nitrate N) values, which in general show a gradual decrease with the age of the plants, do not seem to bear any correlation with the NRA.

### SUMMARY

A study of the nitrate reductase activity (NRA) and nitrate N levels at different stages of growth in the stem of 4 sugarcane varieties, has indicated that during the 7th, 8th and 9th month stages of growth of the plants, the observed nitrate N and NRA levels show a good degree of linear relation. Based upon this relation, it has been shown that nitrate N that is potentially significant (pN) in reduction could be calculated at the different stages of growth of the plants. A higher level of pN during the 7th, 8th and 9th month stages of growth in a cane variety appears to be indicative of the superiority of the variety in respect of yield.

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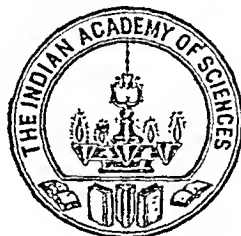
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# STUDIES ON THERMO-PHOTO-SENSITIVITY OF THE PADDY PLANT UNDER FIELD CONDITIONS\*

BY R. VENKATARAMAN

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(Communicated by Dr. R. Subrahmanyam, F.A.Sc.)

## INTRODUCTION

PHOTOPERIODIC effect on rice has been studied by several workers and reviewed by Ghose *et al.* (1960) in India and Morinaga (1954) in Japan. However, the effect of light on the plant could not so far be generalised. It is accepted that photoperiodic sensitivity itself is subject to temperature (Oka, 1959) but this aspect has not been given due regard in the field (Sampath, 1961). Suenaga (1936) was the first to report on the requirement of the optimum day length of a variety and minimum vegetative growth for flower-initiation in relation to a certain temperature. He, however, did not provide an explanation for the reaction norm. The observation was not also verified objectively under field conditions of exposure.

The present investigation reports a study by statistical methods on the sensitivity of the paddy plant to temperature and day length under field conditions of exposure. Information is provided on (i) characteristic effects of day length and temperature on the plant, (ii) optimum requirement of day length and temperature of the plant, (iii) sensitivity of the plant to deviations of the above from optimum and (iv) variability of development within a clump, due to different states of sensitivity of the shoots during growth.

## MATERIAL

The observations were carried out at the Agricultural Research Station, Palur, South Arcot District, Madras State (Latitude 11° 45' N., Longitude 79° 40' and Altitude 44' M.S.L.). Ten varieties of paddy, two late, three medium and five early, were transplanted, when the seedlings were 30 days

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\* Published with the approval of the Director of Agriculture, Madras State.

old, at ten fortnightly intervals The details of treatment and lay-out are given below

1 *Manures*

2,270 Kg green leaf + 68 Kg. superphosphate + 34 Kg Ammonium sulphate applied basally before transplantation

2 *Varieties*

Late PLR 1 and Co 19

Medium ASD 5, ASD. 11 and Co 30

Early TKM 6, TKM. 3, PLR. 2, ADT 20 and Co 29

3 *Planting time*

No	Planting date	No	Planting date
I	1-7-1961	VI	16-9-1961
II	16-7-1961	VII	1-10-1961
III	1-8-1961	VIII	16-10-1961
IV	16-8-1961	IX	1-11-1961
V	1-9-1961	X	16-11-1961

4 *Day length and temperature*

Day length was calculated from the almanac of South Arcot District for 1961-62 Temperature is that absolute maximum daily recorded at the Agricultural Research Station, Palur, at 8 A.M every day

5 *Lay-out*

- (1) *Planting*. Singles in row 10' apart and 6' between plants in the row
- (2) *Number of rows*. Six including two guard rows
- (3) *Total units under observation* 10 (varieties)  $\times$  10 (plantings) = 100

6 *Cropping condition*

The crop was raised under wet conditions, adequately irrigated from time to time. The field was kept free from weeds

### METHOD

Observations were recorded from random samples of clumps collected from the middle four rows Twenty clumps were sampled for each of the hundred units under study. The characters studied were (1) height of each tiller in the clump (measured from base to tip of earhead), (2) ear-length against the height of each tiller, (3) number of functional tillers in each clump, and (4) duration from sowing to flowering in each unit of observation.

Observations (1) to (3) were recorded following the harvest and (4) on the date of flowering.

Fortnightly means of corresponding values of day length and temperature were correlated to establish the association of their occurrence.

Day length and temperature, being factors of weather that give combined effect on plant characters, mentioned above, it was necessary to work partial correlations to determine their separate effects. Again, as the object was to study general reactions, the means of group values of the late, medium and early varieties on each plant character were considered in the correlation studies. The stage or stages at which induction takes place was deduced from significant correlations between quantitative attributes of plant characters and the weather factors, for periodical intervals from planting to flowering. Data on plant characters from I to X plantings on one side and on the other, those of the corresponding day length and temperature for every fortnight separately from planting to flowering were correlated. In other words, the values of each plant character of I to X plantings were kept fixed and the values of day length and temperature were slid up against them and every shift gave a new set of ten pairs of values. The different partial correlation coefficients on the combinations were calculated limited up to the tenth, eighth and fourth fortnight from planting respectively for the late, medium and early groups of varieties and the limit reached the flowering time. One hundred and seventy-six calculations were made.

The measurements of height and ear-length of tillers from all the clumps, for each planting of a variety, were grouped in respective correlation tables and the correlations were tested. These calculations are based on a total of 9576 independent pairs of observations.

## RESULTS

### *(a) Correlation between Natural Day Length and Temperature*

The data collected on day length and temperature are presented in fortnightly means in Table I. The coefficients of correlation between day length and temperature are presented in Table II. Table I shows that both day length and temperature decrease from July to middle of December (Fortnights I to XI) and increase from December to June (Fortnights XII to XXIV). Table II establishes highly significant positive correlation between the two weather factors, while indicating possibilities of slight disturbances in their relations due to season.

TABLE I

*Fortnightly mean values of day length and temperature  
(Palur, 1961-62)*

Fort- night No	Begin- ning date	Day length		Tempe- rature °C	Fort- night No	Begin- ning date	Day length		Tempe- rature °C
		Hr	Min				Hr	Min	
I	1-7-1961	12	41	33.2	XIII	1-1-1962	11	20	26.9
II	16-7-1961	12	37	33.8	XIV	16-1-1962	11	26	27.2
III	1-8-1961	12	30	32.9	XV	1-2-1962	11	30	27.7
IV	16-8-1961	12	21	33.2	XVI	16-2-1962	11	40	29.6
V	1-9-1961	12	12	33.3	XVII	1-3-1962	11	48	30.0
VI	16-9-1961	12	04	32.2	XVIII	16-3-1962	11	56	31.3
VII	1-10-1961	11	50	32.6	XIX	1-4-1962	12	05	33.0
VIII	16-10-1961	11	43	30.4	XX	16-4-1962	12	19	34.0
IX	1-11-1961	11	33	29.7	XXI	1-5-1962	12	29	34.4
X	16-11-1961	11	24	27.2	XXII	16-5-1962	12	35	34.8
XI	1-12-1961	11	19	25.1	XXIII	1-6-1962	12	39	35.4
XII	16-12-1961	11	18	26.6	XXIV	16-6-1962	12	42	36.8

*(b) Quantitative Attributes of Plant Characters*

The data on the quantitative attributes of the four plant characters against each time of planting are presented in Table III.

1. *Height*—Height decreased from planting II to planting VII in medium and late groups and up to planting VI in early group. In the subsequent plantings, there was tendency for increased growth. However, in planting IX, height decreased once again.

2. *Ear-length*—The length of the earhead increased up to planting III in late group, planting IV in mixed urn group and planting II in early group. There was reduction in length in the subsequent plantings, till planting VII in all the groups. There was tendency to increase again from planting VIII.

TABLE II

*Simple correlations between mean day length and temperature*

Sequence of fortnights from Table I		Correlation Coefficients ( <i>r</i> ) (Significant at $P = 0.01$ )	Conditions of influencing weather
Months	Fortnights (Ten at a time)		
July to November	.. I to X	0.8748	Summer
Do.	.. II to XI	0.8923	
August to December	.. III to XII	0.9097	
Do.	.. IV to XIII	0.9397	
September to January	.. V to XIV	0.9484	N.E. Monsoon
Do.	.. VI to XV	0.9298	
October to February	.. VII to XVI	0.9572	
Do.	.. VIII to XVII	0.9230	
November to March	.. IX to XVIII	0.9348	Spring
Do.	.. X to XIX	0.9783	
December to April	.. XI to XX	0.9833	
Do.	.. XII to XXI	0.9931	

though followed after a fall in planting IX. The fall continued in the early group.

3. *Tillering*.—The data relate to functional tillers. Tillering was low against planting I and increased up to planting IX in late group and planting VIII in medium group. In early group, tillering was high against planting I, fell up to planting VI and increased again.

4. *Duration*.—Duration was delayed on either side of planting VII for late and medium groups and planting VI for early group.

(c) *Partial Correlation Coefficients between Plant Characters and Temperature Eliminating Effect of Day Length*

The coefficients of partial correlation of temperature separately with the four plant characters, for the late, medium and early groups of varieties, with reference to age of the crop are presented in Table IV.

TABLE III  
*Mean values of characters of early, medium and late varieties*

Period/ Varieties	Height (cm)			Ear length (cm)			Tiller per clump (No)			Flowering duration (No of days)		
	Late	Medium	Early	Late	Medium	Early	Late	Medium	Early	Late	Medium	Early
I	148.7	146.2	91.5	25.2	24.5	21.3	2.4	3.7	5.5	164	148	65
II	152.7	153.9	101.6	25.7	23.8	21.8	2.3	2.8	5.0	158	142	66
III	145.5	150.2	91.0	25.9	27.1	21.1	3.0	2.9	4.6	149	134	64
IV	132.8	141.1	95.9	23.0	27.4	20.6	4.7	4.8	4.4	143	123	63
V	122.7	139.2	89.4	22.2	24.2	19.1	4.7	4.4	4.3	138	118	62
VI	120.7	131.5	86.3	22.0	23.9	19.6	4.9	5.2	3.5	132	113	62
VII	99.5	94.4	69.4	20.7	20.2	19.9	5.9	5.6	5.1	125	111	68
VIII	105.5	98.4	89.4	22.8	23.4	19.3	7.1	7.0	5.9	127	116	73
IX	91.5	86.2	81.1	21.3	21.6	18.8	8.2	4.8	5.7	130	117	76
X	105.5	108.3	78.6	21.9	22.0	18.2	5.5	5.1	5.4	134	119	77

TABLE IV  
Partial correlation coefficients with temperature

Fortnight/ from planting/ varieties	$r$ 15.6			$r$ 25.6			$r$ 35.6			$r$ 45.6		
	Late	Medium	Early	Late	Medium	Early	Late	Medium	Early	Late	Medium	Early
First ..	-.6049*	-.4588	.4682	-.5986	-.1684	-.2805	.1069	.2015	-.3329	-.6621	-.6191	-.6726†
Second ..	-.2862	-.0270	.3001	-.4061	.2849	-.1564	.2579	.6135	-.5283	-.0601†	-.8351*	-.8204†
Third ..	.2072	.5905	.3062	-.2816	.2356	-.4764	.2957	.1939	-.6907*	-.6761†	-.7647*	-.8379†
Fourth ..	.3506	.6662	.1353	-.4594	.4409	.0000	.4201	-.2120	-.9522†	-.3111	-.6612	-.6056
Fifth ..	.4877	.5666	..	.5169	.8196†	..	.3649	-.3056	..	.4248	.3616	..
Sixth ..	.0040	.6907*	..	.5564	.9154†	..	.2422	-.0190	..	.1469	.4169	..
Seventh ..	.3934	.2264	..	.4264	.2760	..	.1277	-.5387	..	.3161	.3987	..
Eighth ..	.9410†	.0073	..	-.2090	-.1381	..	.4569	-.3927	..	.3746	.4019	..
Ninth ..	.1564	..	..	.1114	..	..	.1371	..	..	.1220	..	..
Tenth ..	.2774	..	..	.2315	..	..	.2372	..	..	.2828	..	..

\* Significant at  $P = .05$ .† Significant at  $P = .01$ .



1. *Height*—In late group significant negative correlation in the first fortnight and positive correlation in the eighth fortnight from planting were recorded

The trend of association was negative against the first two fortnights from planting for late and medium groups and positive later. It was positive for the early group of varieties

2 *Ear-length*—For the medium group, coefficients against the fifth and sixth fortnights from planting were positive and highly significant.

The trend of association was negative against the first four fortnights for the medium group and first three fortnights from planting for the early group. The remaining trends were positive

3 *Tillering*—For the early groups, the coefficients against the third and fourth fortnights from planting were negative and significant.

The trend of association was completely negative for the early group, negative from the fourth fortnight for the medium group and completely positive for the late group

4 *Duration*.—All the significant correlations were negative and were recorded against the first to third fortnights for medium and late groups.

The trend of association was negative up to the fourth fortnight for the late and medium groups of varieties and positive later. It was completely negative for the early group

(d) *Partial Correlation Coefficients between Plant Characters and Day Length Eliminating Effect of Temperature*

The coefficient of partial correlation of day length separately with the four plant characters, in respect of the late, medium and early groups of varieties, with reference to the age of the crop are presented in Table V.

1. *Height*—All the significant correlations were positive and were recorded against the first four fortnights from planting for the late group and first two fortnights for the medium group

The trend of association was completely positive for the early group and up to the sixth and fourth fortnights from planting respectively for the late and medium groups. The trend against the seventh and eighth fortnights for the late group and fifth, seventh and eighth fortnights for the medium group was negative. The ninth and tenth fortnights from planting for the late group and the sixth fortnight for the medium group tended to positively associate with day length

TABLE V  
Partial correlation coefficients with day length

Fortnight from planting/ varieties	$r$ 16.5			$r$ 26.5			$r$ 36.5			$r$ 46.5		
	Late	Medium	Early	Late	Medium	Early	Late	Medium	Early	Late	Medium	Early
First ..	.9197†	.8296†	.3943	.8271†	.5572	.4335	—	.2502	—	.5198	.8254†	—
Second ..	.8768†	.6966*	.3509	.7266*	.1675	.7745*	—	.2616	—	.9223*	.9292†	.3132
Third ..	.8086†	.3908	.2711	.6711*	.1686	.7966*	—	.2532	—	.6469	.9215†	.512
Fourth ..	.7313*	.1217	.2463	.7630*	.1361	.6673*	—	.2619	—	.7274*	.8804†	.3313.
Fifth ..	.3796	—	.0570	.0538	.7121*	..	—	.3695	—	.9035†	.7617*	..
Sixth ..	.3011	.2413	..	.2273	.6989†	..	—	.5219	—	.7681*	.5977	..
Seventh ..	—	.2942	—	.1570	.2503	..	—	.6010	—	.7429*	.4774	..
Eighth ..	—	.3357	—	.4222	.2214	..	—	.0675	—	.1042	—	.1163
Ninth ..	.1271	..	..	.0812	..	..	..	.1076	..	..	..	..
Tenth ..	.3840	..	..	.4129	..	..	..	.3361	..	..	..	..

\* Significant at  $P = .05$ .† Significant at  $P = .01$ .

2. *Ear-length*—All the significant correlations were positive and were recorded against the first four fortnights for the late group, fifth and sixth fortnights for the medium group and second to fourth fortnight for the early group

The trend of association was completely positive in all the three groups of varieties

TABLE VI

*Significant effects of day length and temperature on plant characters*

MEDIUM VARIETIES			LATE VARIETIES			EARLY VARIETIES		
Temperature effect	Age in fortnights from planting	Day length effect	Temperature effect	Age in fortnights from planting	Day length effect	Temperature effect	Age in fortnights from planting	Day length effect
	1 2 3 4 5 6 7	$\frac{Ht}{(+)}$ $\frac{D}{(-)}$ $\frac{T}{(-)}$		1 2 3 4 5 6 8	$\frac{Ht}{(+)}$ $\frac{D}{(-)}$ $\frac{EL}{(+)}$		1 2 3 4	$\frac{D}{(-)}$ $\frac{EL}{(+)}$ $\frac{T}{(+)}$
	$\frac{Y}{X}$			$\frac{Y}{X}$				$\frac{V}{R}$

\* This may be read that height is positively correlated to day length in the first two fortnights and accelerates. The other notations may also be read similarly

Ht = Height

EL = Ear length

T = Tillering (functional)

D = Duration

(+) = Hasten or accelerate

(-) = Delay or retard

V/R = Line dividing vegetative period at the top and reproductive period below

3. *Tillering*.—For the medium group, the coefficients were significantly negative against the second and fourth to seventh fortnights from planting and positive for the early group against the fourth fortnight.

The trend of association was completely positive for the early group, completely negative for the medium group and negative up to the seventh fortnight from planting and positive later for the late group.

4. *Duration*.—All the significant coefficients were positive and were recorded against first to fifth fortnight for the late and first to sixth fortnight for the medium groups.

The trend of association was positive except against the eighth fortnight for the medium and late groups and first fortnight for the early group.

*(e) Stages of Life of the Plant in Relation to Significant Association with Temperature and Day Length*

The associations of plant characters of the late, medium and early groups of varieties with day length and temperature were traced on the same stage of life phase of the plant. The significant partial correlations of day length and temperature extracted from Tables IV and V are presented side by side in Table VI to facilitate discussion. The responses, due to temperature and day length, varying for the vegetative and reproductive phases of the plant appear to be essentially similar for all the three groups of varieties.

The vegetative phase of the plant continues till flower-initiation. According to Oka (1958) flower-initiation is expected to occur about 30 days before flowering. In this study the minimum duration to flower for the late group is 125 days and for the medium group is 111 days. Deducting 30 days, the flower-initiation may be expected to be about the fifth fortnight from planting (95 days from sowing) for late group and fourth fortnight from planting (81 days from sowing) for the medium group, and not earlier. That is, up to these limits the vegetative phase continues. As regards the early varieties, it is observed from *Reports of the Agricultural Stations in Madras* (1933), that flower-initiation commences much earlier to cessation of vegetative activity of the clump. Hence, vegetative activity continues till end or three fortnights from planting. Thus the line V/R in Table VI formally demarcates the vegetative and reproductive stages of life of the plant in the group.

*(f) Correlation between Height and Ear-length*

Table VII presents the correlation coefficients between height and ear-length within the clump. Of the total of 100 correlation coefficients, 50

TABLE VII  
Correlation coefficients between height and ear-length

Variety/ Period	Late			Medium			Early			
	PI R. 1	Co. 19	ASD. 5	ASD. 11	Co. 30	TKM 6	TKM 3	PLR 2	ADT 20	Co. 29
I	.037	.027	.003	.026	.128	.572†	.727†	.235	.384†	.465†
II	.061	.202	.045	.418†	.312*	.364†	.434†	.607†	.307†	.720†
III	.075	.228	.195	.124	.094	.725†	.702†	.742†	.465†	.497†
IV	.483†	.257*	.287†	.357†	.193	.526†	.545†	.584†	.391	.230†
V	.388†	.149	.163	.164	.500†	.203	.406†	.709†	.042	.293†
VI	.359†	.302†	.056	.123	.257†	.164	.312†	.497†	.175	.842†
VII	.790†	.138	.102	.022	.036	.553†	.464†	.093	.102	.191
VIII	.214†	.460†	.262†	.491†	.102	.314†	.668†	.471†	.270*	.472†
IX	.591†	.665†	.428†	.257†	.362†	.068	.203	.340†	.536†	.054
X	.018	.285†	.377†	.345†	.142	.000	.381†	.148	.509†	.509†

\* Significant at P = .05.

† Significant at P = .01.

were recorded from the early group and the other 50 from the medium and late groups. 61 correlations were significant. Of these, 37 belonged to the early group and the balance 24 to the late and medium duration groups. Further, associations of the value,  $r = 0.500$  and above, were 17 out of 37 in the early group and only 3 out of 24 in the other two groups.

## DISCUSSION

Only the significant correlations are interpreted for general discussion and the non-significant trends compliment them in contexts. Positive correlation signifies positive effect on the development of plant character following increased day length or temperature. Negative correlation signifies negative effect on the development of plant character following increased day length or temperature. By positive effect is meant increase in height, ear-length and number of functional tillers and reduction in the number of days to flower. In the same way, by negative effect is meant reduction in height, ear-length and number of functional tillers and increase in number of days to flower.

Duration provides an estimate of the average rate of development. From Table III, it could be seen that the flowering duration of any group of varieties, including the early group, varies with time of planting. For instance, for the late group the duration extended from 125 to 164 days. In other words, flower-initiation being 30 days before flowering, the plant can attain full development for flower-initiation by growing for 95 days only or more up to 134 days, depending on planting time. In the former instance, the growth period is shorter and development is faster ( $1/95$ ) while in the latter instance the growth period is longer, but rate of development is slower ( $1/134$ ). Growth refers to increase in the size and number of cells, while development refers to their progressive state in time towards differentiation of stages in the plant. Growth is measured in space, while development is measured both in space and time. Oka (1958) recognised the two different processes in paddy plant.

### (a) *Disposition of Natural Day Length and Temperature*

From Table I, it may be seen that rise and fall of the temperature, in season, depend on the position of the earth in relation to the sun. From Table II, it may be seen that the two factors are invariably closely associated. In the sequences beginning with the summer months (July–August) the magnitude of the correlation values is slightly less. The values improve in the sequences beginning with the monsoon months (September–November). The relations are good from close of winter to spring (December–April).

The orderly and slight influences of the environment, and the high degree of the correlation and its stability in the sequences, from any part of the year, practically affirm that day length of a season has its own selective temperature and *vice-versa*

*(b) Time and Effect of Induction of Day Length and Temperature*

Table VI shows that (1) temperature produces the negative effect on duration while day length produces the positive effect; (2) temperature produces the negative effect on height in the pre-flowering phase while day length produces the positive effect and (3) temperature produces the negative effect on tillering in the vegetative phase of early varieties, while day length produces the positive effect. The above effects of temperature opposed to those of the day length, were noted in the vegetative phase of the plant. So, these are bound to influence the effect of day length on the physiological functions of the plant. The effects of temperature and day length were together positive on ear-length and height at the post-flowering phase. This aspect will be considered separately.

An increase of temperature in the vegetative phase arrests growth and promotes development while increase of day length promotes growth and arrests development. Both growth and development are necessary to complete life-cycle. According to Lysenko (1932; *vide* Chandrasekaran *et al.*, 1953) growth and development are independent and are controlled by different sets of environmental factors. He explained it by the phasic development of the plant. He mentioned the thermo-phase and the photo-phase of the plant and treatments in the order, to the best cultural advantage. He, however, conceded that growth and development can also be concurrent. From the present investigation, it is seen that since temperature and day length produce opposite effects, a balance, physiological in nature, is attained in the plant, at an optimum level for full development with minimum growth. Desired advantages of extended growth can also be produced at adjusted levels of temperature and day length at a time.

*(c) Optimum Requirement of Day Length and Temperature for the Plant*

As stated above, when temperature and day length neutralise each other's effect in the plant, the plant grows under non-sensitive conditions. That is, at this condition, there is only development and no luxurious growth over a minimum. Typical retardations on duration are noted in this study (*vide* Table III) on either side of planting VI to VII for late, medium and early groups of varieties. The growth period up to flowering from these critical

plantings are the least for the respective groups. Therefore, the varieties approach and pass through a brief state of optimum level of temperature and day length obtaining under field conditions. Suenaga (*vide* Morinaga, 1954) observed that at a certain temperature, each variety of paddy had its own optimum day length under which it comes to earing at the earliest. As the day length deviates from the optimum to either the longer or shorter side, the time of ear-emergence is retarded. The present evidence corroborates his observations. Further, each temperature at a time is equated by one complementary day length only in nature. The plant that chooses either of the factors invariably has chosen both as criterion for minimum growth. Thus, optimum temperature and day length of a variety are simultaneously met with under field conditions.

It must, therefore, be possible to find the limits within which the optimum temperature and day length lie for each variety. As, at this level, the reactions are neutralised, it is evident that the level is chosen by the variety itself, and may be called the optimum temperature (or day length) of the variety. The critical planting for early varieties for minimum duration under field conditions is planting VI (*vide* Table III) and temperature has significant influence on duration up to the third fortnight from planting (*vide* Table VI). The temperature of the succeeding third fortnight (Fortnight VIII) is  $30.4^{\circ}\text{C}$ . and of the second fortnight (Fortnight VII) is  $32.6^{\circ}\text{C}$  (*vide* Table I). Hence it can safely be said that the level lies between these two readings. In the same way, for late and medium duration groups, temperature influences duration on the succeeding second and third fortnights and stepping from the critical planting VII, the level can be said to lie between the readings  $29.7^{\circ}\text{C}$ . (Fortnight IX) and  $30.4^{\circ}\text{C}$ . (Fortnight VIII).

Temperature and day length, as recorded in this study, are indices respectively of the total warmth and intensity of light in the day. The effect of natural day length, as a component of temperature, is not only for the duration of sunshine but also for its intensity. The importance of the intensity of light cannot be minimised (Best, 1959). Hence, day length, controlled artificially or compared between different altitudes at same latitude, would need, in effect, correction for intensity.

*(d) Deviation of Natural Day Length and Temperature from the Optimum and Norm of Reaction*

From the above, resultant effect of natural day length and temperature is neutral, when their levels are the same as the optimum for the variety. When that environment is not the optimum for the plant, the development



is delayed. In other words all day length and temperature higher or lower than the optimum give delaying effect. The higher levels are known as super-optimal and the lower levels as sub-optimal.

By virtue of natural day length and temperature (Table I) the super-optimal higher temperature and longer day length go together. Here the delaying effect would appear to be due to the longer day length, for, higher temperature would only hasten flowering as seen from statistical deductions (*vide* Table IV). On the other hand when sub-optimal lower temperature and shorter day length go together in the season the delaying effect would be due to lower temperature for shorter day length would only hasten flowering (Table V). Hence the norm of reaction by which the day length displayed the final effect of delay in the former case and did not do so in the latter case has to be explained. Morinaga and Kuriyama (1954) recorded that the internal mechanism of retardation seemed to be quite different in the case of sub-optimal photoperiod and super-optimal photoperiod.

It would mean that while both temperature and day length decide the rate of development one of them i.e. temperature acts as a modifier of sensitivity and day length gives the momentum (Oka 1959). In the present instance for the late group the optimal day length is about 11.33 hrs. and the optimum temperature 29.7° C. when the minimum growing period was recorded against planting VII. Earlier plantings under super-optimal levels of temperature and day length delay heading. A probable explanation is that the higher temperatures increase metabolic activity of the plant. The effect due to longer day is expressed better and heading is delayed. The higher the level the higher was the variation from the minimum. In the later plantings sub-optimal and decreasing temperatures depress metabolic activity. The hastening effect of sub-optimal in decreasing day length is minimised resulting in delayed heading. In short the hastening or delaying effect of day length is enhanced by increasing temperature and suppressed by decreasing temperature. The combined effect, therefore is due to thermo-photo-sensitivity.

The retardation which is so conspicuously noted for flowering duration, has no parallel for height and ear length (Table III). It is seen that the height of plant and ear length could be reduced by decreasing day length even though duration was prolonged under sub-optimal conditions. This is yet another aspect of day length (*vide* Table VI). Significant correlations in the vegetative phase with regard to height and ear length are found only with day length and not with temperature except in one instance for height in the medium group. This aspect of day length may be due to the photo-

synthetic action of light, which is indistinguishable experimentally from the primary effect of light (Mohr, 1962).

*(e) Effect of Field Exposure in Relation to Time of Planting*

Any variation in the treatment alters the normal processes of growth and development at a stage when the plant responds to induction. Under field conditions, Ramiah (1933) recorded that the duration of a medium duration variety varied from 90 to 230 days depending on the time of planting. But, the limit to acceleration is the absolute minimum vegetative period (M.V.P.) required for flowering (Suenaga, *l.c.*). This results by growing the plant under its optimal conditions of day length and temperature for a definite and continuous period. Field exposure is not so uniformly regulated. The minimum for a variety, met with under field conditions, is just an approximation and above the real (M.V.P.). The former can vary from place to place. Therefore, a plant under field conditions can be made to ear earlier, by treatment, at any part of the year.

The late and medium groups of varieties grow under super-optimal conditions at planting I (Table III). From planting I onwards temperature and day length decrease gradually in the growing period and approach the optimum for the groups by the eighth fortnight. This seasonal convergence to optimum level may be called the convergent exposure. Under sub-optimal conditions, the divergence of day length and temperature from the optimum increases in the season. Hence, this may be called a divergent exposure.

Between July and December, the optimal condition for all the three groups of varieties was observed to have occurred only once. However, the rise of temperature and day length from December onwards points to the possibility of growing in a convergent exposure and attaining optimal levels for a second time between February and April. Thus, it is possible to cultivate an early variety economically twice in a year, in the summer and in the spring. A late or medium variety has two selective planting seasons in the year at Palur. Under such circumstances, every variety has a selective adoption to season, in relation to its optimal conditions. All varieties appear to be weather-bound. All of them are relatively insensitive at the optimal conditions and sensitive at other states.

A plant may grow completely in either state of convergent or divergent exposure, with reference to its optimum. It may also pass from any one state to the other gradually during the growing period, owing to the time of planting. The rate of development is continuously altered. Convergent exposure gives gradually increased accelerating effect and divergent exposure

gradually increased retarding effect on development. Therefore, the effects of field exposure on a plant varies widely with time of planting.

(f) *Effect of Field Exposure in Relation to Induction State of the Plant*

The above varied situations of field exposure interfere with experimental results of partly controlled treatments. Induction stage of the plant under field conditions, in the present study, extended from the 45th day to 75th day for medium and late groups and 30 days onwards from sowing for the early group. This period may be reduced on account of short-day treatment.

Misra (1954) gave short-day treatment for six weeks to week-old seedlings of medium varieties. Heading was delayed. When the treatment was continued till heading, heading was hastened. Ghose and Shastri (1954) gave 8 hrs. (day length) treatment for 20 days or 30 days old seedlings of a number of varieties. Heading was hastened in all instances. Sircar and Ghose (1947) reported delay in heading in summer varieties of paddy by short-day treatment to the seedlings for four weeks and high temperature treatment for first ten days.

The above results may be interpreted in the following manner. In Misra's trials, the treatment was stopped too early, while the plant could yet respond to induction. Due to the initial treatment, development was accelerated. When the treatment was over and the plant came under longer day length of field exposure, it was actually in a higher metabolic state than the control. As a result, heading was delayed. The continuance of the treatment to heading prevented response to an alternate field exposure thus resulting in early flowering. The author has explained the results suggesting that in the ontogeny of the crop, there was a "reversal of the delaying effect to earliness". In the trials of Ghose and Shastri (*l.c.*), the treatment period was postponed and covered the stage when the plants could respond to induction. The results were uniform. In the experiments of Sircar *et al.* (*l.c.*) again, the treatment ended early. The plant could be induced further, when longer day length of the field exposure played on the higher metabolic state of the treated plant and delayed heading. The authors, however, interpreted the results that short day did not induce earliness, but 'devernalised' the effect of high temperature.

(g) *Variations in the Expressions of Growth within a Clump*

The data on correlation coefficients shown in Table VII illustrate the result of unsteady relations in growth between height and ear-length. This may be due to the fact that for the same height, the corresponding length of

earhead from different shoots varied very much within the clump, in respect of each planting. The variations were partly due to induction effects and partly to direct effect of day length as already discussed. The effect varied for each shoot, as each shoot, from its time of origin developed under different field exposures.

It may be noted that in the early group of varieties, correlation between height and ear-length was much better. The values were high. During the limited growth period of 45 days from planting to flowering, for this group, the seasonal variation was also limited that gave a rather uniform effect. The less significant associations observed in late and medium groups can be attributed to the longer growth season, and variable effect of field exposure on the plant.

The data from the investigation lead to the inference that varieties have to be tested as to their duration and growth in the varied climatic conditions to determine their comparative adaptability and that loss of crop can be avoided by suitably changing the cropping pattern.

#### SUMMARY

The results of a study on the thermo-photo-sensitivity of the paddy plant of different varieties under different field conditions are reported.

The flowering duration of paddy varieties of the early, medium and late groups varied with time of planting. The particular planting in which the duration to flowering is minimum represented the critical planting time. Conditions of day length and temperature of the growing period at that planting are close to the optimum level of requirement of day length and temperature for the variety. The method of deciding the level from data is explained. Earlier or later plantings than the critical resulted in delayed heading.

The reaction norm of sub-optimal, optimal and super-optimal day length and temperature, on the plant, is explained. All varieties have selective adoption to season and are weather-bound. All of them derive insensitive and sensitive conditions of growth from the environment. The study shows that naming varieties as "period-bound" or "season-bound" cannot be strictly justified.

The main features of field exposure and its variable effect on a growing plant from season to season and within a clump is described. The reasons for variability of results of controlled treatments are discussed. It is also

found that the height of the shoot has no direct attributive influence on its ear-length

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# ON A NEW SPECIMEN, PROBABLY OF *PALMOXYLON SUNDARAM* (SAHNI) FROM MOHGAON KALAN, MADHYA PRADESH

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## INTRODUCTION

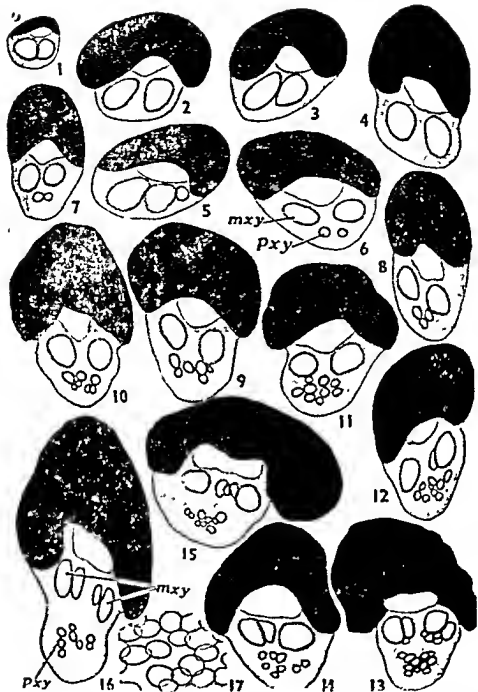
THE present paper deals with a piece of petrified wood from the Deccan Intertrappean beds in Madhya Pradesh and is comparable to Prof. Sahni's (1946) *Palmoxylon sundaram* described from the same locality. His description of this species was based upon a reconstruction made after piecing together a number of fragments distributed in different museums in India. This fragment discovered in 1951 while conforming to the species *sundaram*, showed many variations from the type species. In view of this it was thought desirable that the specimen should be described. The fuller significance of this will be discussed later.

## DESCRIPTION

The specimen described here is fragmentary and brownish-white in colour. It measures about 3.5 cm. in diameter and 10.5 cm. in length (Photo 1). Transverse sections show that the tissue cannot be divided into different zones as in several other species of *Palmoxylon*. The distribution, structure, arrangement and frequency of the vascular bundles indicate that the piece probably belongs to the subdermal or central zone of a palm wood.

## ANATOMY

The distribution of the fibrovascular bundles in the only zone recognisable in the specimen, is irregular throughout (Photo 2). The vascular bundles are of different sizes and shapes (Figs. 1-8). Most of them are bigger ones but in between them are found some slightly smaller ones also (Fig. 1). They are usually rounded to oval in shape (Figs. 1-3, 5 and 6). Some are elongated also (Figs. 4, 7 and 8). Their frequency is 80-100 per cm.<sup>2</sup>, F/v ratio is 0.5/1-1/1 and their diameter is 0.2-0.6 mm. The dorsal sclerenchyma is



TEXT-FIGS. 1-17. Fig. 1 One small fibrovascular bundle in the transection of the wood,  $\times 80$ . Figs. 2-5 Different kinds of fibrovascular bundles in the transections with 2-3 metaxylem vessels,  $\times 72$ . Figs. 6-8 Vascular bundles showing metaxylem as well as protoxylem vessels,  $\times 72$ . mxy, metaxylem; pxy, protoxylem. Figs. 9-14 Different types of leaf-trace bundles in the transverse section of the wood,  $\times 72$ . Fig. 15 One compressed leaf-trace bundles,  $\times 72$ . Fig. 16 A leaf-trace bundle showing tongue-like process of the vascular part,  $\times 72$ . mxy, metaxylem; pxy, protoxylem. Fig. 17 A part of the ground tissue in the transection of the wood,  $\times 80$ .

Dermal sclerenchyma solid black, phloem white patch, parenchyma dotted.

of different forms (Figs. 1-8). But generally they are of reniform type (Figs. 2-4, 7 and 8; Photo 3). The auricular lobes are poorly developed. The median sinus is rounded to concave (Figs. 2-8). The sclerenchyma cells are arranged closely without intercellular spaces and are pentagonal to hexagonal in nature (Photo 3). The phloem is preserved only in a few bundles. The vascular part is bigger than the fibrous part in most of the cases, sometimes equal to it and consists of the xylem vessels and parenchyma. The metaxylem vessels (*mxy*) are generally two, round to oval, lying side by side, excluded (Figs. 1-8; Photo 3). The protoxylem (*pxy*) is quite clear (Figs. 6-8; Photo 3). The xylem vessels are surrounded by parenchyma but no posterior sclerenchyma is present. There is no tissue developed around the fibrovascular bundles as radiating or tabular parenchyma. There are no fibrous bundles either. Stigmata are absent around the fibrous part of the fibrovascular bundles.

Leaf-traces are seen throughout the wood. They vary in sizes and shapes (Figs. 9-16). Some are very big (Figs. 9, 10, 13-16) and the others are of median sizes (Figs. 11 and 12). In the leaf-trace bundles the sclerenchyma part is shorter than the vascular part and are quite different in form (Figs. 9-16). The median sinus is round and the auricular lobes are round to pointed. The dorsal sclerenchyma in some of the leaf-trace bundles is rather compressed and is not of any definite shape (Fig. 15). The vascular part consists of 2 or 3, sometimes, a row of (*mxy*) metaxylem vessels and a number of (*pxy*) protoxylem vessels (Fig. 16 and Photo 4). In some cases the vascular part projects far out of the median sinus as a tongue-like process (Fig. 16 and Photo 4). But in others they are broad (Figs. 10-15). There is no posterior sclerenchyma in the leaf-trace bundles also.

Ground tissue consists of lacunar cells rounded to elliptical, small, more or less uniform in size, with thin smooth walls (Fig. 17). The cells surrounding the fibrovascular bundles are tangentially flattened. In longitudinal sections the cells are seen to be placed in vertical rows.

The pitting of the metaxylem is multiseriate scalariform (Photo 5) and the pitting of the protoxylem is spiral. The perforated end walls show parallel bars of thickening.

No leaf-bases or roots are seen in this specimen.

#### DISCUSSION AND COMPARISON

Of all the species of *Palmoxylon* described from India, listed by us (Rao and Menon, 1964) *P. sundaram* is the only one with which our specimen



TABLE

Characters	<i>P. hislopi</i> Rode (1933)	<i>P. kamalam</i> Rode (1933) and Shukla (1939)
Frequency	80-120/cm <sup>2</sup>	90-100/cm <sup>2</sup> } Rode 25-28/cm <sup>2</sup> in the external region 10-12/cm <sup>2</sup> in the middle region } Shukla 22-25/cm <sup>2</sup> in the central region
F/v ratio	7/8	2/3-1/2 } Rode 8/1-10/1 in smaller bundles } Shukla 3/1-4/1 in bigger bundles
Diameter	0.6-0.8 mm	0.25-0.45 mm
Dorsal sclerenchyma	Rounded, oval or semicircular with a concavity either narrow and deep or broad and shallow where it receives the vascular part	Oblong to ovate with a flat complanate type of base where the vascular part joins the fibrous part. Mostly not preserved leaving a large central cavity within a thin fibrous lining
Ventral sclerenchyma	Absent	Absent
Xylem vessels	Usually 2	2-3
Fibrous bundles	Absent	Absent
Stigmata	Absent	Absent
Radiating or tabular parenchyma	Radiating parenchyma present, tabular parenchyma absent	Radiating parenchyma present, tabular parenchyma absent
Ground tissue	A network of narrow elongated cells enclosing larger polygonal intercellular spaces	A network of narrow, elongated parenchyma cells. Just midway between the neighbouring bundles the parenchyma cells get compressed into linear dark regions which serve to divide the whole ground mass into distinct compartments

I

<i>P. arcotense</i> Ramanujam (1953)	<i>P. sundaram</i> Sahni (1946) Sub-dermal zone	The specimen described in this paper
D-110/cm. <sup>2</sup> SD-50-65/cm. <sup>2</sup> C-20-25/cm. <sup>2</sup>	SD-100/cm. <sup>2</sup>	80-100/cm. <sup>2</sup>
D-15/1 SD-5/1-8/1 C-2/1-3/1	3/2-1/1	0.5/1-1/1
D-50-100 $\mu$ SD-175 $\mu$ C-100/200 $\mu$	1/2-1 mm.	0.25-0.6 mm.
Orbicular to reniform	Lunate and reniform type. Round median sinus	Reniform type with round to concave median sinus
Absent	Present in leaf-trace bundles	Absent
2	2	Mostly 2. Proto-xylem present
Absent	Present	Absent
Absent	Present	Absent
Absent	Absent	Absent
Lunate with network of narrow rectangular cells often of varied shapes	Regular pattern of small, loosely packed smooth-walled elliptical cells of uniform size. The cells surrounding the fibrovascular bundles are more or less markedly compressed in a tangential direction	Lunate with loosely packed smooth-walled elliptical cells more or less uniform in sizes. The cells surrounding the fibrovascular bundles are more or less compressed in a tangential direction

D, Dermal zone; SD, Sub-dermal zone; C, Central zone.

can be compared in some detail. On the basis mainly of its lacunar ground tissue it offers comparison with *Palmaxylan blanfordi* Schenk (Schenk, 1882, Stenzel, 1904), *P wadlai* Sahni (1931), *P mathuri* Sahni (1931), *P jammuese* Sahni (1931), *P sundaram* Sahni (1946), *P hislapi* Rode (1933), *P kamalam* Rode (1933) and Shukla (1939), *P arcotense* Ramanujam (1953), *P dakshinense* Prakash (1958), *P chhindwareense* Prakash (1958) *P eacenum* Prakash (1961) and *P parthasarathyi* Rao and Menon (in press). On the basis of this and some other characters also a closer comparison is possible only with *P hislapi* Rode, *P kamalam* Rode, *P areatense* Ramanujam and *P sundaram* Sahni. This comparison is shown in Table I.

Table I further shows that our specimen as already stated can be compared closely only with *P sundaram*. Yet, it differs from it in the following characters. There are some peculiarly constructed diminutive bundles and fibrous bundles present in the sub-dermal and central zones of *P sundaram*. They are completely absent in our specimen. The presence of stigmata is conspicuous in *P sundaram* but they are not present in the specimen described here. The  $f/v$  ratio of the fibrovascular bundles even though comparable in both the specimens, is slightly higher in *P sundaram* than in our specimen. It is  $3/2-1/1$  in *P sundaram* and  $0.5/1-1/1$  in our specimen. The lunate type of dorsal sclerenchyma and 'the "trivial character" of the tendency for the phloem to become bilobed' as in *P sundaram* are not seen in our specimen. Lastly, the ventral sclerenchymatous are capping the protoxylem of the leaf-trace bundles in *P sundaram* is also not clear in the specimen described in this paper.

In view of the above differences of a rather important nature our specimen cannot be placed in *P sundaram* unless the original diagnosis of the species is changed. At the same time the original specimen is much bigger, complete and has furnished a number of characters. Our specimen is rather fragmentary and may or may not really be identical with *P sundaram*, nor has it yielded sufficient number of distinguishing characters to justify its specific separation. We therefore think that provisionally at least this specimen should not be put into a new species but can be placed as a new variety of *P sundaram*.

Like *P sundaram* our specimen can also be accommodated in the section *Cocos* like palms and subsection *Rentfarmia* of the combined scheme of Von Mohl (1849) and Stenzel (1904).

*Palmoxyton sundaram* SAHNI VAR. *Vidarbhai* RAO AND MENON

Diagnosis

Fibrovascular bundles irregularly orientated and variously shaped; 80–100 per cm.<sup>2</sup> rounded to elongated in form; F/v ratio 0.5/1–1/1; diameter 0.25–0.6 mm.; dorsal sclerenchyma mostly reniform; median sinus rounded to concave, auricular lobes poorly developed, sometimes rounded; metaxylem vessels usually two; protoxylem vessels present. No fibrous bundles and stegmata. Radiating and tabular parenchyma absent around the vascular bundles. Leaf-trace bundles present. Ground tissue lacunar with cells elliptical more or less same in size with smooth thin walls. The cells surrounding the fibrovascular bundles tangentially flattened.

SUMMARY

A piece of petrified palm wood from Mohgaon Kalan resembling *P. sundaram* Sahni has been described in this paper. Although it resembles *P. sundaram* in most of the characters, yet this specimen shows some important variations from *P. sundaram*. It is, therefore, placed provisionally as a new variety of *P. sundaram*.

Locality—Mohgaon Kalan area.

Age—Eocene.

Type specimen—M2 (Kept in the Botany Department, Lucknow University).

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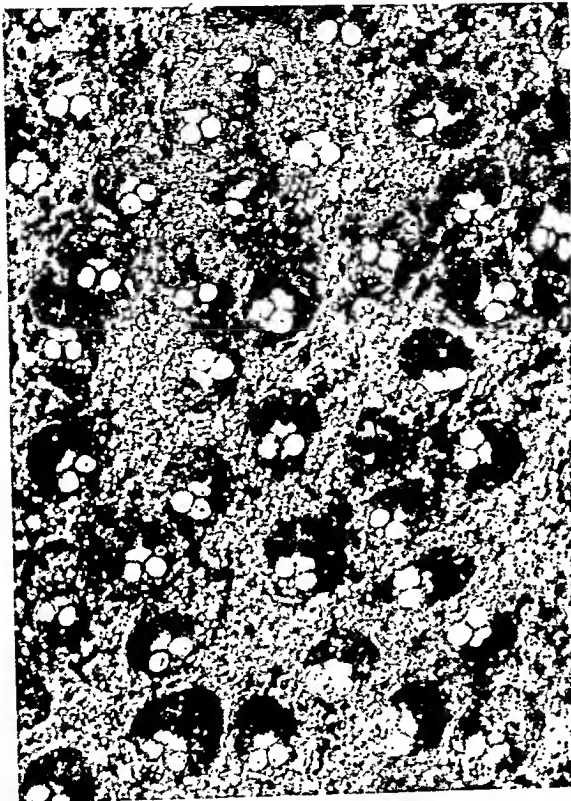
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## EXPLANATION OF PHOTOGRAPHS

## PHOTOS 1-5

- PHOTO 1. Specimen of palm wood before sectioning,  $\times 0.46$
- PHOTO 2. Part of transverse section showing the distribution of the fibrovascular bundles,  $\times 12$
- PHOTO 3. A single fibrovascular bundle enlarged,  $\times 146$
- PHOTO 4. One enlarged leaf-trace bundle,  $\times 162$
- PHOTO 5. Longitudinal section of part of wood showing multiserrate scalariform pitting of the vessels,  $\times 302$ .



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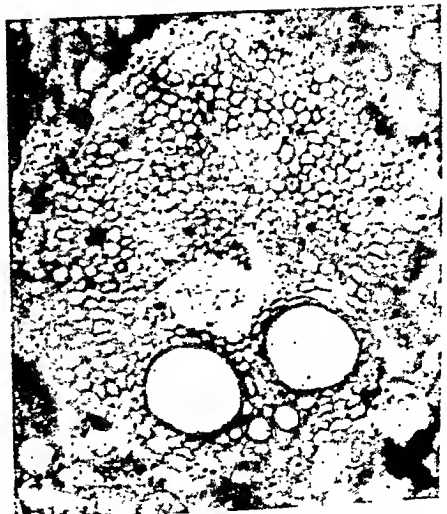
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# INFLUENCE OF pH AND TEMPERATURE ON THE OXYGEN CONSUMPTION OF THE EARTHWORM, *LAMPITO MAURITII*

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## INTRODUCTION

INVESTIGATIONS on the effect of sudden changes in pH of the medium on the metabolism of animals are almost non-existent except for two papers, by Tang (1936) on the yeast cells and by Ramamurthy (1964) in crabs. This may be due to the fact that organisms do not usually come across wide fluctuation in the pH of the medium. But this is of interest in the worms, where the soil pH in which the worms live may change due to the decaying matter and may also vary with the temperature changes in the seasons.

## MATERIAL AND METHODS

Earthworms of the species *Lampito mauritii* were collected from the fields round about Tirupati. They were kept in the laboratory, submerged under water with blotter pulp, in glass troughs covered with wire-mesh. Water was changed daily once. After 2 or 3 days, by which time the muddy content in the gut of the worms was replaced by the blotter pulp, measurements were made by Winkler's method (Saroja, 1959).

To measure the respiration in relation to pH and temperature, 4 different pH media 4, 6, 8, 9.5 and four temperatures 20°, 25°, 30° and 35° C. were used. pH 3.5 and 10 were lethal to the worms. Measurements were made at one temperature per day at pH 4, 6, 8 and 9.5 in an ascending order, starting first at 20° C. To get the required pH, dilute HCl (0.2 N) or NaOH (0.2 N), as the case may be, was added to the tap-water and the pH was accurately measured by using the Beckman pH-meter. The pH of the water was measured daily which ranged from 6.85 to 6.9 all through the time of the experiments. The pH of the water in the respiration chamber was measured after the experiment to see whether it changed due to the CO<sub>2</sub> liberated by the worms during the experiments. There was a change to alkaline side when chambers of glass other than pyrex were used. Hence only pyrex

bottles of 125 ml capacity were used for these experiments. The pH of the body fluids of the worms kept at extreme pH (4 and 9.5) and temperature (35° C) was measured using the narrow range pH papers.

### RESULTS

Oxygen consumption is measured in *Lampito mauritii* at various temperatures in media of different pH and the results are given in Figs 1-16. The regression lines of the Figs 1-16 are compared in Figs 17-24.

*Influence of pH on the Oxygen consumption at various temperatures*—Irrespective of the pH of the medium the Oxygen consumption of the worms is relatively constant at 25° C and 30° C but for the little depressing effect in the larger individuals at pH 4 and 8 respectively (Figs 5-12 and 18-19). The overlapping regression lines in Figs 18 and 19 indicate that this insensitivity to the wide range of pH round about habitat temperature (28° C.) is the same throughout the size range. It is of interest to know that at 25° C. the Oxygen consumption of the worms in the natural medium (tap-water, pH 6.85) is also similar to that observed at other pH.

It can be seen from Figs 17-20 that at temperatures around the normal (room) changes in metabolism due to pH are not observed, while extreme temperatures seem to elicit differential response to the varying pH of the medium in the Oxygen consumption. At 20° C and 35° C not only the metabolic rate changes in response to changing pH, but the magnitude of this response appears to vary in small, as compared to the large individuals and this is reflected in the different slopes (*b*) of the regression lines (Table I).

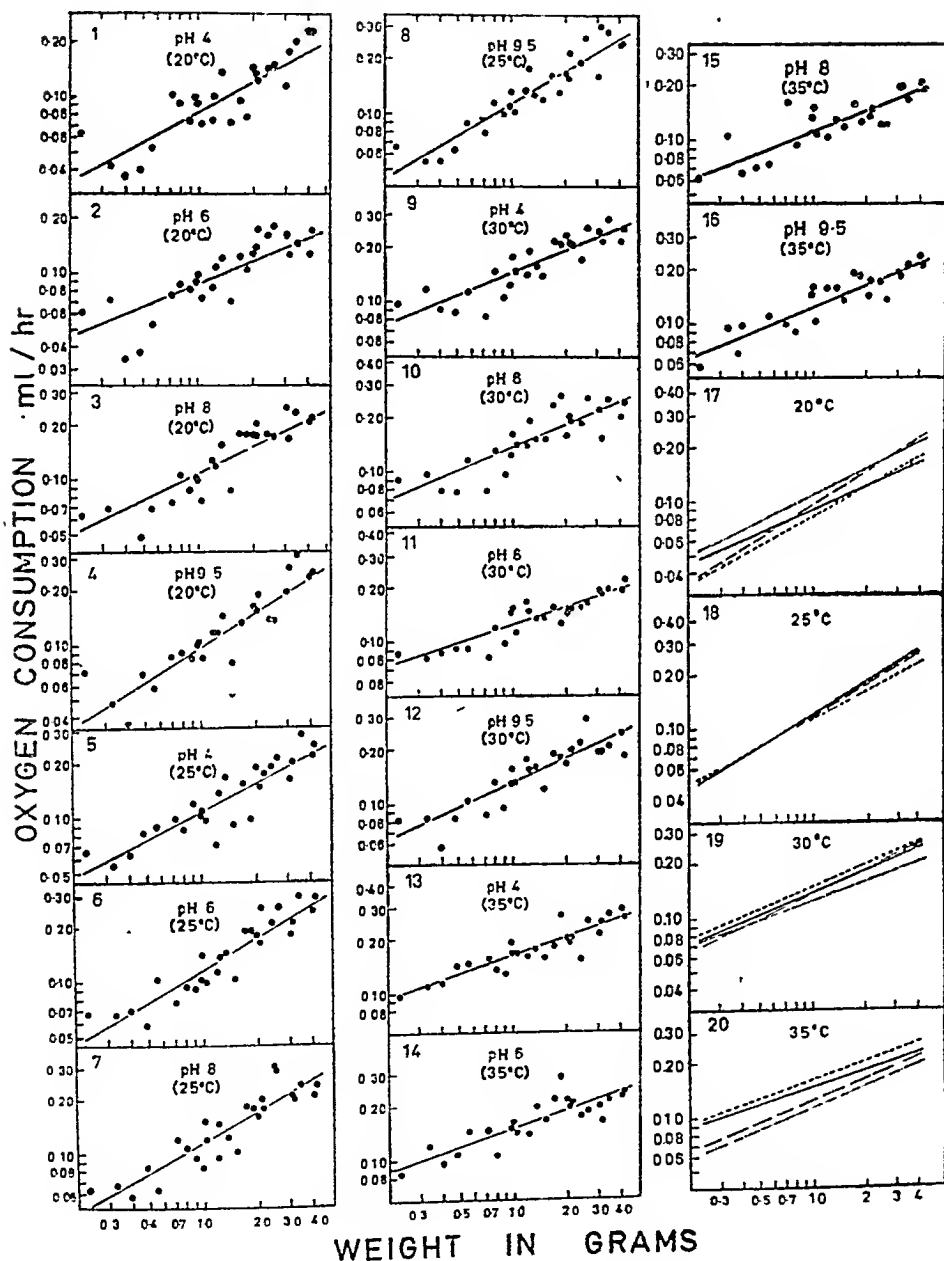
TABLE I

*Weight regression (b) values as a function of temperature and pH*

The values at pH 6.85 (tap-water) in all the tables are taken from the published data (Saroja, 1959)

pH	Temperature ° C.			
	20	25	30	35
4.0	0.545	0.515	0.414	0.339
6.0	0.412	0.584	0.411	0.316
6.85	0.745	0.628	0.623	0.560
8.0	0.432	0.565	0.346	0.394
9.5	0.618	0.583	0.44	0.402





FIGS. 1-20. Figs. 1-16. Oxygen consumption of *Lampito mauritii*, under water, as a function of pH and temperature. Figs. 17-20. Oxygen consumption—size regression lines of *L. mauritii* in media of different pH and temperatures. Dashed line pH 4; Unbroken line pH 6; Dash and dot pH 8; Broken line pH 9.5.

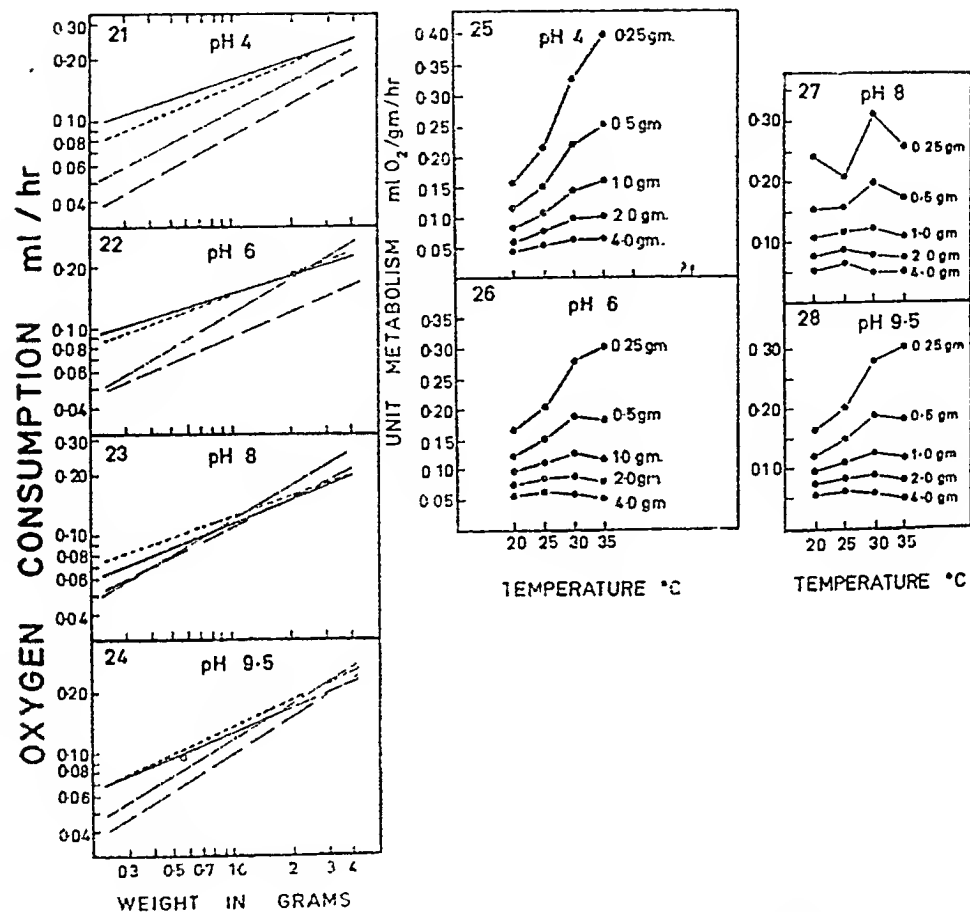
Not considering the  $b$  in the natural medium, the higher  $b$  values are obtained at the extreme pH media (4 and 9.5) at 20° C and in the alkaline medium at 35° C

Maximal and minimal consumption at 20° C is observed at pH 8 and 4 respectively, though this is reversed in the extreme (larger) size range, by having maximal and minimal consumption at pH 9.5 and 6 (Fig. 17). It is of interest to note that the reverse trend is seen at 35° C, where maximal consumption is observed at pH 4 and minimal at pH 8 in the order of 4, 6, 9.5 and 8. Thus consumption in the acid medium is higher than in the alkaline medium and this effect of acidity and alkalinity is the same in all sizes.

Table I clearly demonstrates that  $b$  in the natural medium is greater than those on either side of this pH 6.85, at any temperature. The metabolic rates of the smaller individuals weighing below 1 gm are higher at all pH media and temperatures (resulting in low  $b$  values) except at 35° C where the metabolic rate is higher in all the size groups in the natural medium than in the alkaline medium which shows the inhibition of metabolism of the worms at this extreme high temperature as already mentioned.

The afore-mentioned results, when compared to the results of the natural medium, show that the smaller individuals consume more Oxygen (except in the alkaline media at 35° C) when the pH of the medium is altered from the natural value. Similarly, a change in pH to either side of the natural medium inhibits the metabolism of the larger individuals. This effect of varying pH is negligible at 25° C and not very significant at 30° C, which are near the habitat temperature (28° C).

*Influence of pH on the temperature response of Oxygen consumption*—Figures 21-24 demonstrate that Oxygen consumption increases with increasing temperature with a few exceptions within the pH range studied. The upward shift of the regression lines with increasing temperature is not parallel. This differential effect of temperature on size is clear from the different  $b$  values at each pH as given in Table I. In the lower size ranges the temperature response is greater between 25° and 30° C in all the pH media. At pH 4 and 9.5 the regression lines are parallel below the room temperature (28° C). Metabolism of the larger individuals is more depressed at 30° and 35° C in all the pH media. In general at 25° C (where consumption is independent of pH) the  $b$  values are higher as seen in Table I. It is evident that the Oxygen consumption in different pH media at the temperatures nearing the habitat temperature is not surface dependent and especially above habitat



FIGS. 21-28. Figs. 21-24. Oxygen consumption of size regression lines of *L. mauritii* at different temperatures in media of different pH. Dashed line 20° C.; Dash and dots 25° C.; Broken line 30° C.; Unbroken line 35° C. Figs. 25-28. R-T curves of *L. mauritii* as a function of size in different pH media.

temperature *b* values are very low as can be expected from the depressed metabolism of the larger worms.

It is worthy of note that at alkaline pH the regression line of 35° C. lies below that of 30° C. showing that the high pH has got depressing effect on metabolism at that high temperature.

These trends can be clearly understood from temperature coefficients given in Table II and from the unit metabolism of different size groups at various pH plotted in Figs. 25-28 against temperature.

R-T curves show that response to temperature is greater in smaller worms as shown by their steeper slopes. At the pH media below the natural pH

(6.85) R-T curves of all sizes show an increase with temperature except at the temperature range 30°-35° C at pH 4 in the larger individuals (above 2 gm). The same trend is to be seen at pH 6 in the 4 gm individual where the depression occurs at 30° C itself. At pH 8 this trend is reversed in relation to size; i.e., the metabolic depression is greater in the smaller worms between 30° and 35° C (Fig. 27). Two gm and 4 gm individuals show a decrease in their metabolic rate between 25° and 30° C. Figure 28 indicates that the trends at pH 9.5 are similar to pH 8 except that there is no heat depression in the smaller individuals (0.25 gm).

It is evident from these R-T curves that the rate of increase in Oxygen consumption is lower in alkaline media when compared to acid media. This is more clearly brought about if  $Q_{10}$  is considered. Table II shows that the response to temperature decreased with increasing pH except in the natural pH media. In alkaline pH, the negative  $Q_{10}$  values at high temperatures indicate that the metabolism is inhibited by the combined effect of high temperatures and high pH. This decrease in  $Q_{10}$  on either side of the natural pH demonstrates that change in pH of the medium affects the temperature sensitivity of the worms to a considerable extent and this is manifested to a greater degree at high pH.

No trend is to be seen in  $Q_{10}$  with temperature.  $Q_{10}$  decreased with weight except in pH 6 where it increased with weight between 20° and 25° C. and in pH 8 where it decreased with weight only between 25° and 30° C. No change was observed in the pH of the body fluids of the worms kept for an hour at the extreme pH (4 and 9.5) and temperature (35° C.).

TABLE II

*$Q_{10}$  of Oxygen consumption as a function of size and temperature  
in media of different pH*

Weight gm.	pH 4	pH 6	pH 6.85 (tap-water)	pH 8	pH 9.5
	20-25 25-30 30-35	20-25 25-30 30-35	20-25 25-30 30-35	20-25 25-30 30-35	20-25 25-30 30-35
0.25	1.83 2.35 1.49	1.04 2.15 1.49	1.96 1.84 1.64	0.75 1.77 0.68	1.47 1.89 1.18
0.5	1.78 2.10 1.35	1.40 1.74 1.31	1.26 1.52 1.61	1.04 1.60 0.74	1.44 1.60 0.84
1.0	1.63 1.83 1.23	1.7 1.37 1.22	1.39 1.51 1.35	1.15 1.12 0.80	1.33 1.33 0.99
1.8	1.70 1.60 1.34	2.02 1.17 1.11	1.2 1.51 1.47	1.24 0.92 0.83	1.26 1.18 0.84
2.0	1.64 1.58 1.54	2.17 1.07 1.11	.. .. .	1.27 0.82 0.83	1.31 1.07 0.97
4.0	1.85 1.31 1.0	2.77 0.85 0.97	.. .. .	1.5 0.57 1.0	1.25 0.91 0.75

## DISCUSSION

Though a few papers are available on the study of the influence of pH upon the rate of Oxygen consumption, no direct data are available regarding the relation of temperature to the amount of Oxygen consumed at media of different pH.

Ramamurthy (1964) showed that in the crab *Paratelphusa*, respiration varied directly with pH in the smaller individuals and in alkaline medium the increase was parallel throughout the size range studied. But his studies are restricted to one temperature. Tang (1936) has shown that the respiration of yeast cells at different Hydrogen-ion concentrations was influenced by the nature of the buffer systems used, because buffer solutions affect the respiration in two ways, the pH of the solutions and their ionic composition. Using phosphate buffer he measured temperature coefficients of respiration at 23–31° C. in the media of pH 5.9 and 6.9. He showed a very slight increase in  $Q_{10}$  at pH 6.9.

Several species of fishes have the ability to extract Oxygen from water at low Oxygen tensions equally well over a fairly wide range of Hydrogen-ion concentration (Power, 1931; Pruthi, 1926 and Wiebe *et al.*, 1934).

Hiestand (1931) had shown that low pH increased Oxygen consumption in dragon-fly larvae.

The data of the present investigation show that in natural temperature the worms are able to regulate their rate of consumption over the wide range of pH studied. It has been observed that pH 3.5 and 10 are lethal to the worms as they died within half an hour in those media at the habitat temperature itself. It is an established fact that organisms could tolerate wide ranges of pH, this being ascribed to the fact that hydrogen and hydroxyl ions penetrate very slowly. Moreover, this slow penetration facilitates neutralisation of these by the buffering system that is present in the organism.

The lack of sensitivity to pH, and the constancy of the pH of the body fluids in different pH media that are observed in the earthworms, explain the presence of a perfect buffering system. Yet it is astonishing to note that the worm is able to regulate its respiration up to the lethal pH on either side in its habitat temperature.

The effect of pH on the rate of Oxygen consumption by worms in the acid range is different from its effect in the alkaline range below and above the room temperature at which the worms were kept (27° C.). As it is already shown, the effect of pH on the worms at normal temperature is

negligible. The effect at different temperatures must be only due to the influence of pH on temperature response of the worms, and this is most pronounced at the highest (35°C) and the lowest (20°C) temperatures studied.

These data indicate a cumulative effect of temperature, Oxygen tension and pH of the medium on the metabolic rate of the worms. At low temperature where the tension is at its highest, low pH seems to inhibit rate of respiration. But at pH 4 the response to temperature is similar to that of normal pH (6.85). At 35°C where the Oxygen tension is low, the high pH inhibits the respiration affecting the activity of the animal so much that it consumes less at 35°C than at 20°C.

The low  $Q_{10}$  at different pH when compared to the natural pH (6.85) shows that somehow the change in pH is affecting the magnitude of the response to temperature. This decreased sensitivity to temperature is greater at the higher hydrogen ion concentration especially at the high temperature range. The low  $b$  values also show that size has got more influence on Oxygen consumption as pH of the medium changes on either side of the normal pH and the  $b$  decreases as temperature increases in the natural and extreme pH ranges studied. This also shows that smaller and bigger worms are affected in opposite directions by change in pH (except at 35°C in the alkaline media), increasing the rate of respiration in the smaller worms and decreasing in the larger worms at all the temperatures studied except at 25°C. This differential but opposite effects of pH on small *versus* large individuals is difficult to interpret.

But this metabolic regulation over the extraordinary wide range of pH at and around the habitat temperature till it reaches the lethal pH on either side must be of survival value to the earthworms as they have to live and thrive on the decaying matter which changes the pH of the soil in which they exist.

### SUMMARY

Combined effect of the pH and temperature showed that the respiration of *L. mauritii* is independent of pH of external medium round about habitat temperature.

### ACKNOWLEDGEMENT

My grateful thanks are due to Professor Kandula Pampapathi Rao, Head of the Department of Zoology and Principal, Sri Venkateswara University, for suggesting this problem and for his invaluable guidance throughout.

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# SOME SHORT-PERIOD CHANGES IN THE ATMOSPHERIC SPORE CONTENT ASSOCIATED WITH CHANGES IN THE WEATHER AND OTHER CONDITIONS

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## INTRODUCTION

THAT the changes in the weather conditions affect spore content of the atmosphere both qualitatively and quantitatively has been recognised long ago. As early as 1872, in the course of his microscopic examinations of the air in Calcutta, Cunningham (1873) observed how rainfall brings about short-period changes in the atmospheric spore content. Although Miquel (1883) attempted to study some of these short period changes, he could not provide any precise information for want of a suitable air sampling equipment. The introduction of the automatic volumetric spore trap, designed by Hirst (1952) for continuous air sampling with which the time of deposition of any particular spore on the trace can be determined accurately, has made it possible to undertake such studies. Hirst (1953) using this trap, for the first time, described the alterations in the major constituents of the air-spores of an arable field at the Rothamsted Experimental Station Harpenden U.K. associated with rain, thunder-shower, dew formation, etc. Short period changes observed in some of the common constituents of the air-spores at Visakhapatnam in India which are associated with some factors like rainfall, flooding of the area with very heavy rains, harvesting operations, etc. are described in this paper.

## METHODS

By operating the Hirst trap with its orifice at 1.75 metres above the ground level changes in the different constituents of the air-spores of a paddy field near Visakhapatnam were studied continuously over a period of more than two years. The methods followed in conducting air sampling with the Hirst trap, procedures adopted in mounting and scanning of the slides exposed



in the trap were in general the same as those described by Hirst (1953) and Sreeramulu and Seshavatham (1962). The slides were scanned at 2-hourly intervals. Separate counts were taken for the different categories (whose identification was based on visual features alone) listed below: *Spores identified up to the species level: Piricularia oryzae, Trichoconis padwickii, Helminthosporium oryzae, Deightonella torulosa and Corynespora cassiicola. Spores identified up to the generic level: Cladosporium, Fusarium, Nigrospora, Periconia, Curvularia, Alternaria, Helminthosporium* type (except *H. oryzae*), *Cercospora* (counted under two categories—'short' type in which spores below  $80\mu$  in length were included and 'long' type for those above  $80\mu$ ), *Tetraploa* and *Phaeotrichoconis*. *Heterogeneous groups: Basidiospores, Aspergilli, Ascospores, Bunts, Uredospores of rusts, fragments of hyphae and pollen grains.* All those not included in any of the above-named groups were counted under one category—the 'Unclassified' for estimating the total air-spores. From the counts, assuming the efficiency of the trap as 60%, the number per cubic metre of air was estimated.

To illustrate some of the representative short-period changes from the results of this two-year survey, six periods have been selected which are presented in this paper. In Figs. 1-6 the changes in the estimated concentrations of the different spore types selected in each period are plotted at 2-hourly intervals along with the changes in the weather conditions in that period recorded with self-recording meteorological instruments located in the Visakhapatnam aerodrome.

All times are given in Indian Standard Time (I.S.T.).

## RESULTS

*Changes in the spore concentrations associated with rainfall in the period from 12 to 18 October 1960 in the main crop season.*—This period was selected from the rainy season when the rice crop in the field was passing through the 'maximum tillering phase'. In this period many of the leaf-infecting fungi were showing their seasonal maxima. How rainfall occurring at different times in the day influences the short-period changes in the catches of eleven air-borne spore types common in this period can be seen in Fig. 1.

Dry conditions existed in the daytime on 12 and 13 October although there was rainfall (of 2.2 and 0.3 mm. respectively) in the evenings. Diurnal periodicity patterns of the different spore types were not affected by these rains which occurred in the evening hours.

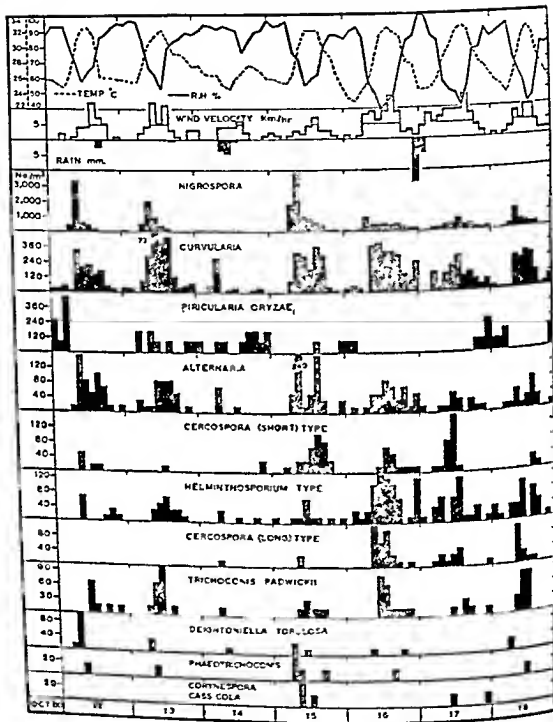


FIG. 1. Changes in the concentrations of eleven air-borne spore types (common in the main crop season) associated with rainfall.

The 14 October was a cloudy day and there was rainfall of 8.1 mm. between 09.00 and 13.00 hr. The relative humidities (R.H.) were above 90% level almost throughout the day. The diurnal periodicities of all the 'day-spore\*' types were affected by this rain and they appeared in very low concentrations on this day. The 'night-spore\*' types (not shown in Fig. 1) dominated the air-spore on this day.

The weather was dry on 15 and 16 October and the diurnal fluctuations in temperature and R.H. were normal for this season. All the spore types regained their normal periodicities and they appeared in concentrations usual in this part of the year.

There was an amount of 18.7 mm. of rainfall between 23.00 and 02.00 hr. in the night of 16-17 October, the time at which the night-spore types generally exhibit their daily maxima. Although this rainfall in the night did not affect the periodicities of the day-spore types (shown in Fig. 1) on 16 and 17 October it affected the concentrations of the air-borne ascospores and basidiospores (not shown in Fig. 1) considerably. Ascospores which were appearing in concentrations of 430/m.<sup>3</sup> at 22.00 hr. before the rain increased to 2,410/m.<sup>3</sup> with the starting of the rain. Basidiospores showed relatively high concentrations (9,200/m.<sup>3</sup>) at 22.00 hr. but their numbers were reduced to 3,700/m.<sup>3</sup> by this rain. Soon after this rain again there was an increase in their numbers (8,200/m.<sup>3</sup> and 14,700/m.<sup>3</sup> at 02.00 and 04.00 hr. respectively).

All the spore types exhibited their regular diurnal periodicities in air on 18 October which was a normal dry day.

From the changes observed on 14 October with rain in the forenoon and those of 16-17 October with rain at midnight (the peak hours of the day-spore and the night-spore types respectively) it can be said that the time of rainfall has a pronounced effect on the numbers of any particular spore type in the air.

*Changes in the spore concentrations associated with rainfall in the period from 5 to 15 February 1961 in the second crop season.*—This period was selected to show the influence of rainfall on the concentrations and the diurnal periodicities of ten air-borne spore types common in the second crop season.

All the ten spore types illustrated in Fig. 2 exhibited their normal diurnal periodicities on 5 and 6 February.

\* Spore types whose daily peak concentrations recur in the daytime showing the *forenoon* and the *afternoon* patterns in Gregory's (1961, pp. 117-19) classification and those with the daily peaks recurring in the night showing the *nocturnal pattern* are referred to as the 'day-spore' and the 'night-spore' types respectively.

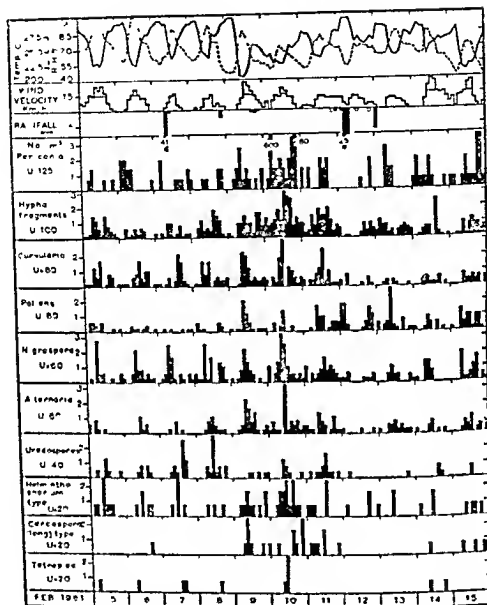


FIG. 2. Changes in the concentrations of ten air-borne spore types (common in the second crop season) associated with rainfall.

On the morning of 7 February there was a rainfall of 42 mm. (between 09.00 a.m. and 12.00 hr). Soon after the rain there was bright sunshine and the weather became dry. The concentrations of all the spore types with their peaks in the forenoon hours were affected by this rain excepting those of *N. griseopora* with its peak (at 08.00 hr) before this rain. Usual high numbers

for spore types like *Curvularia*, Uredospores, etc., were encountered in the afternoon when the conditions became dry. But *Cladosporium* and *Alternaria* occurred in very low numbers throughout this day.

On 8 February all the day-spore types once again showed their regular periodicities in air. A rainfall of 2.4 mm. was recorded in the night between 21.00 and 22.00 hr. During this rainfall high concentrations of ascospores (6,000/m.<sup>3</sup>) appeared and after the rain increase in the catches of basidiospores (10,920/m.<sup>3</sup>) and spores of *Fusarium* (2,060/m.<sup>3</sup>) occurred.

Due to the rain in the previous night, in the morning of 9 February humid conditions prevailed up to 08.00 hr. A sudden decrease in R.H. from 99% to 50% occurred between 08.00 and 09.00 hr. during which period an increase in the wind speed (to 18 km./hr.) was also observed. These rapid changes occurring at the peak hours of spore types with the forenoon pattern account for their high catches at 10.00 hr. on this day. Although there was a rainfall of 2.1 mm. between 18.00 and 21.00 hr. the R.H. values were below 73% throughout the night which were probably due to the prevailing high wind speeds (7-10 km./hr.). Low concentrations of the night-spore types occurred in the night.

High winds were encountered throughout the day on 10 February (daily mean wind speed: 10.8 km./hr.) and the R.H. values were very low. On this day very high concentrations of many of the day-spore types were observed.

The conditions were dry on 11 February and the wind speeds were in the range of 2-17 km./hr. All spore types appeared in high numbers on this day.

The 12 February was a cloudy day with a rainfall of 33.9 mm. (between 03.00 and 08.00 hr. and with traces of rain at 14.00 and 22.00 hr.). The R.H. values were above 83% and the temperatures were low. All the spore types with forenoon pattern of diurnal periodicity were almost absent in the air on this day. An increase in the numbers of ascospores and basidiospores was observed with the morning rain.

A rainfall of 6.4 mm. was recorded at 01.00 hr. on 13 February. Very low concentrations of all the spore types occurred on this day. The low catches on 13 and 14 February indicate the influence of a continuous rainfall in this prolonged wet period (7-13 February). But within two days (by 15 February) most of them recovered their normal periodicities.

The changes observed on 7 and 12 (both days with rain in the morning) indicate that though rainfall in the morning affect the catches of the day-spore types, some of them appear in high numbers in the afternoon if dry conditions prevail immediately after the rain. The effect of high wind speeds and rapid fluctuations in R.H. on the catches is evident in the changes recorded on 9 and 10 February. That in prolonged wet periods the sources of the air-spores get exhausted leading to extremely low catches is also evident from the data of 13 and 14 February.

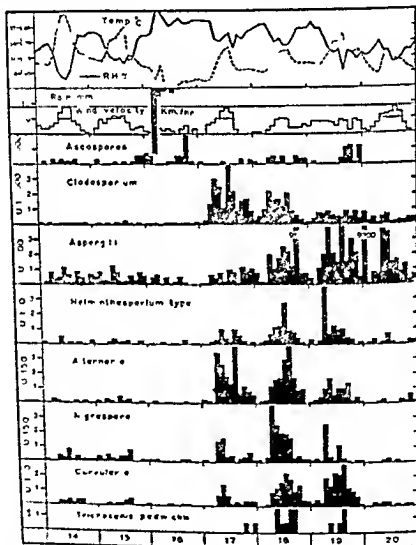


FIG. 3. Changes in the concentrations of eight air-borne spore types associated with first rains of the monsoon.

*Changes in the spore concentrations associated with the first rains of the South-West Monsoon in the period from 14 to 20 June 1960.*—In the months of May and June when the fields are barren and when the conditions are dry seasonal minima for many of the common components of the air-spores are found. But a sudden increase in the numbers of the total air-spores was noticed both in 1960 and 1961 with the first rains of the monsoon. The period from 14 to 20 June 1960 was selected to show how the changes observed in some of the important components of the air-spores in these months were brought about by the first rains of the monsoon.

The monsoon in 1960 started with a heavy rain (73.4 mm.) on 16 June. The daily mean concentrations of the total air-spores observed on 14, 15 and 16 June were 2,150/m.<sup>3</sup>, 2,330/m.<sup>3</sup> and 2,100/m.<sup>3</sup> respectively. After the heavy rain on 16 June, there was a sudden increase to 20,600/m.<sup>3</sup> on 17 June. The daily means recorded on the subsequent four days were: 14,940, 10,700, 9,360 and 9,390/m.<sup>3</sup> of air. From the catches it was found that this sharp increase in the total air-spores was due to the increase in the concentrations of the spores of *Cladosporium*, *Aspergilli* and a few other saprobic fungi, brought into the air by the first rains of the monsoon.

From the 2-hourly catches of the eight spore types shown in Fig. 3, it is clear that *Cladosporium* and *Alternaria* showed a quick response than the rest, as high concentrations of these two spore types were recorded on 17 June itself, while spores of *Aspergilli*, *Nigrospora* and *Curvularia* showed higher numbers on 18 June. On 20 June, excepting the *Aspergilli*, the numbers of most of the spore types returned to the pre-rain level.

*Changes in the spore concentrations associated with heavy rains and consequent flooding of the area in the period from 12 to 25 October 1961.*—An amount of about 1,110 mm. of rainfall was recorded in 1961 of which about 410 mm. occurred in the month of October. On 18 October alone there was a rain of 165.4 mm. This heavy rain flooded much of the area in and around Visakhapatnam town. The fields in the vicinity of the spore trap were submerged up to a height of 0.5 metres above ground for about four hours (12.00 to 16.00 hr.) on 18 October. Although this flood receded in the night damp conditions continued to exist in these fields for another two days. The changes in the concentrations of nine common components of the air-spores observed in the period from 12 to 25 October 1961 were shown in Fig. 4, to show how these heavy rains and flooding of the area influenced their catches.

In this period on 12, 13 and 14 October normal changes in the weather conditions occurred. The concentrations recorded for many of the spore

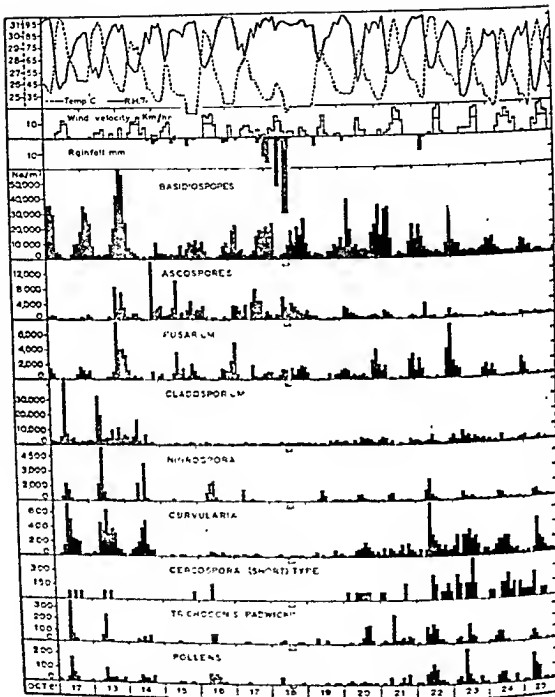


FIG. 4. Changes in the concentrations of nine air-borne spore types associated with heavy rains and flooding of the trapping site. (The period when the field was flooded on 17 October is marked with dotted squares.)



types were normal and all of them exhibited their typical diurnal rhythms on these days.

In the period from 15 to 20 October, there was an amount of 203.3 mm. of rainfall. On 15 October there was a total amount of 16.4 mm. of rain of which 7.8 mm., 4.0 mm., and 4.6 mm. were recorded between 01.00-07.00 hr., 12.00-16.00 hr. and 23.00-24.00 hr. respectively. Due to these showers the day-spore types did not appear in their usual numbers while the night-spore types occurred with higher numbers. On 16 October there was an amount of 4.2 mm. of rain in the early hours of the morning and late in the night. Low concentrations of all the spore types were observed on this day among which only a few types like *Nigrospora* showed their normal diurnal rhythms. October 17 was a humid and cloudy day with 6.5 mm. of rain and further decrease in the day-spore types was observed although the night-spore types were caught in relatively high numbers. An amount of 165.4 mm. of rain fell on 18 October due to which the area was flooded with (0.5 m. deep) water for about four hours (the period of flooding was shown by dotted squares in Fig. 4). On this day all the day-spore types disappeared completely from the air and basidiospores, ascospores and *Fusarium* type occurred in appreciable numbers. October 19 and 20 were days with 6.3 and 3.9 mm. of rainfall. Though certain spore types (like *Nigrospora*, *Trichocontis padwickii*) showed a tendency towards the recovery of their normal diurnal periodicity patterns, their numbers were very low probably because of these rains.

All the spore types reappeared with their normal diurnal periodicities on 21 October which was a dry day. Though an amount of 11.1 mm. of rain was recorded between 06.00 and 08.00 hr. on 22 October, all the spore types showed their regular diurnal periodicity patterns on this day probably because of the return of dry conditions soon after the rain. The 23, 24 and 25 October were normal dry days and all the spore types exhibited their normal diurnal rhythms.

The daily mean concentrations of the total air-spore, the contribution of some of the spore types known to appear under wet conditions ('wet-spore') are given in Table I along with information on the timings and amounts of the rainfall in this period. From the data presented in Table I it is clear that the heavy rains and consequent flooding exerted a great influence on the air-spore of this locality. It is interesting to find that flooding reduced the numbers of even the wet-spore groups to a great extent.

After this damp spell a gradual recovery occurred in the concentrations of the different spore types to the numbers which were existing before the onset of these rains.

TABLE I

Daily mean concentrations of the total air-spores and the contributions of the 'Wet-Spore\*' groups in the period from 12-25 October 1961 along with information on the timings and amounts of rainfall

Date	Time (I.S.T.)	Rainfall Amount (mm.)	Number of spores per cubic metre of air			
			Total air- spora	% of the total air-spora wet in the groups	Mean concentrations for the periods with and without rain	
					Total air- spora	*Wet- spora groups % of the wet in the total air-spora
12	..	0	28,930	18,460	63.12	(Period without rain) 61.61
13	..	0	31,200	18,620	55.98	19,080
14	..	0.6	30,710	20,160	66.65	
15	Throughout the day	16.4	10,960	8,810	80.38	
16	00.00-03.00,					
17	00.00-24.00	4.2	12,410	8,860	71.39	(Period with heavy rain) 77.49
18	17.00, 22.00, 24.00	6.5	16,540	13,300	80.47	
19	Throughout the day	165.4	17,060	13,150	77.67	
20	03.00-10.00	6.3	12,420	8,870	71.42	
21	03.00-10.00	3.9	21,760	14,940	68.94	(Period with moderate rain) 67.84
22	06.00-08.00	11.1	21,020	14,960	71.17	21,330 14,470
23	..	0	22,410	13,520	63.15	
24	..	0	12,660	10,070	44.94	(Period without rain) 50.38
25	..	0	8,860	6,900	54.50	14,550 7,230
				4,710	53.16	

\* Wet up is: Total of basidiospores, ascospores, *Fusarium* and *Phicelaria* only.

*Changes in the spore concentrations associated with heavy dew formation in the period from 9 to 21 November 1961.*—During regular visits to the field it was found that in the period from 7 to 14 November large number of pollen grains were shed and deposited on the leaves of rice plants and other vegetation and on the ground in the vicinity of the trapping site. On all the days between 15 and 20 November, early in the morning, dew formations were observed. The intensity and duration of dew formation in the field on the different days were recorded by taking visual observations. In this period it was also noticed that on the pollen deposits and other decaying matter available in the fields many saprobic fungi grew and sporulated profusely under these humid conditions. Microscopic examinations of these substrata showed that *Cladosporium* was the most common fungus growing on them. Fungal colonies of *Fusarium*, *Nigrospora*, *Curvularia*, *Trichococcus padwickii*, *Alternaria* and others were also found growing on these substrata.

Data presented in Fig. 5 show how these conditions altered the concentrations of some components of the air-spores in the period 9–21 November 1961. Along with the changes in the weather conditions and the concentrations of nine spore forms, the intensity and duration of the dew formation on different days was also shown in Fig. 5 in the form of hatched histograms. The height and the width of these histograms represent the intensity and the duration of dew formation respectively.

In the period from 9–13 November the daily mean concentrations of the total air-spores were in the range of 9,000–15,000/m.<sup>3</sup> During this period high pollen incidence was observed. The weather conditions in these days were dry and there was no dew formation except in the morning of 9 November.

In the period from 15–20 November heavy dew formations were observed in the mornings on all the six days. A gradual increase in the daily mean concentrations of the total air-spores was observed from 17,000/m.<sup>3</sup> recorded on 14 November to 35,400/m.<sup>3</sup> on 21 November. An analysis of the composition of the catches in this period revealed that this increase in the numbers of the total air-spores was mainly due to the increase in the catches of spores of *Cladosporium*, *Fusarium*, *Nigrospora*, *Curvularia*, *Trichococcus padwickii*, *Alternaria* and a few other fungi found growing on the pollen deposits near the trapping site.

According to the previous reports (Sreeramulu and Seshavatham, 1962; Sreeramulu and Ramalingam, 1963) on the air-spores of paddy fields and the observations taken in the present survey in 1960, there should be low incidence of the total air-spores in the month of November. The incidence of

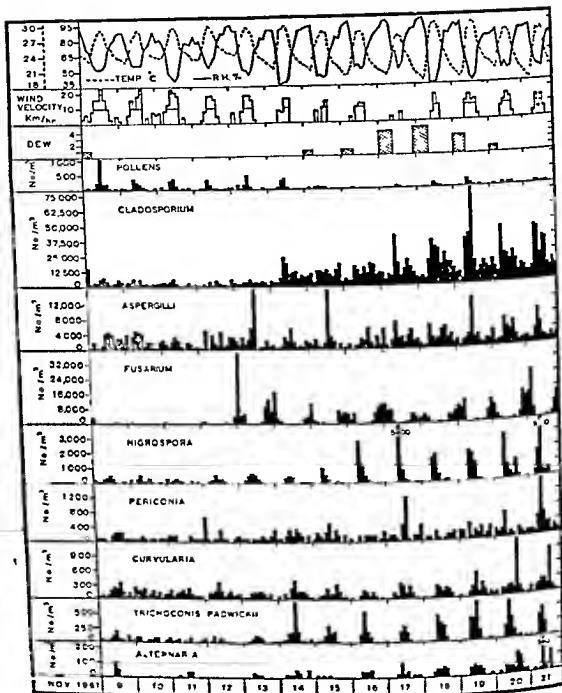


FIG. 5. Changes in the concentrations of nine air-borne spore types associated with heavy dew formations. (The height and width of the histograms drawn for dew indicate the intensity and duration of the dew formation observed on that day.)

very high numbers of the total air-spores in November 1961 was probably due to the high pollen incidence and the heavy dew formation in this period which led to the colonization and abundant sporulation of many saprobic fungi on the substrata available near the trapping site.

*Changes in the spore concentrations associated with the harvesting operations in the period from 2 to 15 December 1960.*—Harvesting of the rice crop in the fields was commenced on 1 December in 1960 in a few plots situated away from the site where the spore trap was located. But these operations were abandoned from 2–5 December as there was rain on 2, 3 and 4 December. Harvesting was carried out on 6 and 7 December in about 2 acres and the crop which was cut on these days was laid flat in the plots. A rainfall of 56 mm. was recorded between 21·00 and 23·00 hr. on 7 December which wetted the standing crop and partly submerged the harvested crop lying flat in the plots for a few hours. Due to this heavy rain harvesting was not done from 8–11 December. On 12 December harvesting in all the remaining plots was completed. The crop in an area of about 2 acres around the trap was cut on this day between 15·00 and 18·00 hr. The collection and removal of the crop previously harvested (which was lying in the field from 7 December) to the threshing floor was also done on this day. These agricultural operations brought about certain interesting changes in the different components of the air-spores. The changes in the 2-hourly concentrations of eight fungus spore types in which the variations observed were most conspicuous were shown in Fig. 6 along with the relevant weather data.

Low concentrations of all the spore types were encountered on 2, 3 and 4 December, the days on which there was rainfall and no harvesting operations were in progress. A considerable increase in the numbers of many spore types was observed on 6 and 7, the days on which the harvesting was in progress. In the night of 7 December there was a heavy rainfall, and reduction in the catches of the different components of the air-spores was observed on 8 December. But on 9, 10 and 11 December relatively high numbers of spores of many saprobic fungi were observed in the air which was probably due to the heavy infection of the harvested crop by saprobic fungi (like *Cladosporium*, *Nigrospora*, *Curvularia*, *Alternaria*, the colonies of which were observed growing in large numbers on the harvested crop) lying flat in the field in these three days. The wet condition of the substrata and the high humidity near the ground level favoured abundant sporulation of these saprobic fungi.

Enormous increase in the numbers of *Cladosporium* and other saprobic fungi and the hyphal fragments were encountered between 15·00 and

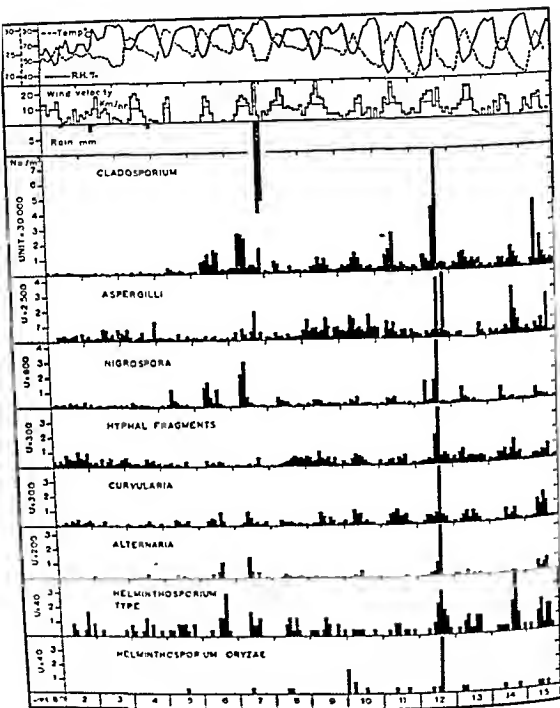


FIG. 6. Changes in the concentrations of eight air-borne spore types associated with the harvesting operations carried on in the fields.

17.00 hr. on 12 December, the time at which the harvesting of the crop in the plots around the trap was in progress. The concentrations of some of the spore types recorded at 16.00 hr. on this day were very high (*Cladosporium*: 240,970/m.<sup>3</sup>; *Aspergilli*: 9,720/m.<sup>3</sup>; *Nigrospora*: 3,550/m.<sup>3</sup>; hyphal fragments: 1,120/m.<sup>3</sup>; *Curvularia*: 1,240/m.<sup>3</sup>; *Helminthosporium oryzae*: 150/m.<sup>3</sup>; *Tetraploa*: 35/m.<sup>3</sup> and *Neovossia*: 35/m.<sup>3</sup>). Basidiospores, Uredospores (and others whose source was not the rice crop) were not at all affected by the disturbances caused by these harvesting operations conducted in the fields.

Relatively high numbers of spores of some of the saprobic fungi were also observed on 15 December at times when collection of the rice crop (harvested on 12 December which was left flat for drying on the ground) was in progress in the plots around the trap.

#### DISCUSSION

The changes in some of the common components of the air-spores at Visakhapatnam in the six periods reported above indicate some of the general effects of the weather and other conditions on the diurnal periodicity patterns of the different spore types and show how the short-period changes encountered in the total atmospheric spore content depended on the variations in the relative contributions of some of the spore types.

Of the different meteorological factors rainfall is found to have a pronounced effect. The influence of rain on the catches can be classified under two heads: *immediate* and *delayed*. To illustrate some of the immediate effects of the rainfall on the catches of certain spore types, the concentrations of fourteen spore types observed *before*, *during* and *after* the occurrence of a rainfall of 21.8 mm. between 10.00 and 14.00 hr. on 28 October 1961 are presented in Table II. The data presented in Figs. 1 and 2 and Table II indicate clearly that rain at the peak hour of any particular spore type has a major influence on its numbers in air. Ascospores and *Fusarium* type show an immediate increase with the starting of rain. Basidiospores show an immediate decrease with rain and reappear in air soon after the stopping of the rainfall. Spore types like *Cladosporium* which are washed off by the first showers of the rain take some time to recover their concentrations to pre-rain levels. Enormous increase in the catches of the spore types exhibiting the forenoon pattern of diurnal periodicity encountered in the forenoons of some days preceded by nights with rainfall (or when there was rainfall on the previous day) might be due to the existence of humid conditions in the preceding night which favour abundant sporulation of these fungi. On

TABLE II

Concentrations of fourteen spore types (number per cubic metre of air) before, during and after rain observed on 28 October 1961

Time (I S T ) Rain (mm )	Before rain	During rain		After rain
	10 00	11 00	12 00-13 00	14 00
		0 4	21 4	
Spore type				
<i>Fusarium</i>	260	260	690	260
Ascospores	260	1,810	35 170	11,950
Basidiospores	430	0	90	2,490
<i>Nigrospora</i>	7,580	310	170	0
<i>Cladosporium</i>	12,560	1,120	1,720	0
<i>Phaeo-riclhoconis</i>	20	0	0	0
<i>Cercospora</i> (long) type	50	0	0	0
<i>Trichoconis padwickii</i>	580	70	20	0
Pollens	30	0	0	0
<i>Alternaria</i>	200	0	0	0
<i>Curvularia</i>	320	50	0	0
<i>Periconia</i>	170	0	0	0
<i>Cercospora</i> (short) type	90	0	0	0
<i>Aspergilli</i>	260	1,120	170	0

certain days following rain in the night or early in the morning high concentrations of certain spore types like *Curvularia* exhibiting the afternoon pattern of diurnal periodicity were also encountered. These are some of the delayed effects of the rain. Meredith (1962) also observed similar effects in the components of the air spora of banana plantations in Jamaica.

The first rains of the monsoon following a long dry spell (Fig. 3), heavy rainfall and consequent flooding of the trapping site (Fig. 4 and Table II) also brought about certain interesting changes in the air-spore



In addition to rainfall excessive dew formations encountered in the month of November 1961 have led to enormous increase in the catches of certain types (Fig. 5), uncommon in this month in the other years.

Harvesting operations in the plots around the trap and collection of the harvested crop left in the fields after drying resulted in an abnormal increase in the concentrations of the air-borne spores of *Cladosporium*, *Nigrospora*, *Aspergilli*, etc. (Fig. 6). Sreeramulu (1958) reported similar increase in the catches of *Cladosporium* and a few other spore types resulting from the mowing of the grass around a barley field.

### SUMMARY

Some short-period changes in the atmospheric spore content which are associated with changes in the weather and other conditions are described. The influence of various factors on the diurnal periodicities in the air-borne spores of many common constituents of the air-spores at Visakhapatnam and variations in their relative contributions to the total air-spores are illustrated from the 2-hourly changes in six periods selected from a 2-year continuous aerobiological survey conducted at Visakhapatnam using a Hirst trap for air-sampling.

### ACKNOWLEDGEMENTS

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# NITROGENOUS MANURING IN RELATION TO BLAST DISEASE OF RICE<sup>1</sup>

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Numerous workers have reported increased incidence of the blast disease of rice, caused by *Piricularia oryzae* Cav., with increased nitrogenous manuring (Sundararaman, 1929; Thomas, 1930, 1938; Suzuki, 1935; Sawada, 1937; Malaguti *et al.*, 1951; Adyanthaya and Rangaswami, 1952; Krishnaswami, 1952; Padmanabhan, 1953). Otani (1952) found that top-dressing of plants with nitrogenous manure resulted in an increase in the  $\alpha$ -amino and amide nitrogen followed by severe infection. Ozaki and Moriyama (1952 *a, b*) stated that glutamine was present in every organ with or without nitrogen, whereas asparagine was absent in plants from the 'no nitrogen plot' during the earlier period of their growth but appeared later in their panicle. Tanaka and Katsuki (1952) reported a low aspartic acid content in rice plants at the time of maximum susceptibility to blast. The total nitrogen content of the leaves and the C/N ratio have been correlated with susceptibility to disease by Yoshii (1941) and Hashiyoka (1944). While there is overwhelming evidence that nitrogenous manuring aggravates the blast disease, no attempts seem to have been made to study the nitrogen status of resistant and susceptible types of rice as influenced by such manuring at various age levels. It is well known that rice plants are particularly susceptible to the disease in the seedling, tillering and earhead stages of growth (Hashiyoka, 1943 *b*; Andersen *et al.*, 1947; Otani, 1953; Padmanathan and Ganguly, 1953). It has also been reported that the resistant types of rice were not affected by heavy nitrogenous manuring (Thomas, 1940; Yoshii, 1941; Krishnaswami, 1952; Padmanathan, 1953). This phenomenon of stability of resistance under heavy nitrogenous manuring can, at best, be understood only by following up the nitrogen pattern of the

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resistant and susceptible types of rice plants. A study of the nitrogen metabolism of the resistant and susceptible rice plants was therefore undertaken, chiefly to assess the nitrogen accumulation in leaves, at various periods of growth including the critical periods of susceptibility as affected by nitrogenous manuring. The total carbohydrates in the rice plant and their consequent effect on the C/N ratio were also investigated.

## MATERIALS AND METHODS

### *Varieties Used*

Two varieties of rice, Co 4 and ADT 10, which have been tested extensively by the State Agricultural Department for their resistance and susceptibility respectively to blast were chosen for the study.

### *Method of Raising Plants*

One part of rice field soil was mixed with an equal part of tank silt, and this mixture was used for raising the seedlings in pots. One series was manured with ammonium sulphate at the rate of 1.0 g per pot (with 4 kg soil) while the other received no manure. Enough water to wet the soil was added daily. When the seedlings were 35 days old they were transplanted in glazed pots (10" x 8" size) containing 5.0 kg of rice field soil to which had been added 20 g green leaves (*Pongamia glabra*) and 1.0 g of superphosphate (19%  $P_2O_5$ ) and which had remained under submerged conditions for 25 days. A day prior to transplantation the soil was thoroughly mixed and ammonium sulphate was added at the rate of 1.25 g per pot (approximately 100 lb/acre) to one series while the other received no nitrogen. Four seedlings were transplanted in each pot. Swampy conditions were maintained throughout the growth of the plant.

### *Collecting Leaf Samples for Chemical Analysis*

Mature leaf samples were collected in the forenoon from quadruplicate pots to minimize sampling errors. The leaves were immediately cut into bits and dried in an oven at 75° C. for 48 hr., ground to a fine powder and stored in airtight vials for analysis. The different stages at which the leaf samples were collected are designated as follows according to the age of the plant—Seedling, tillering, post tillering, shot blade and earhead stages.

### *Analytical Methods*

*Extraction of carbohydrates from plant material*—100 mg samples of the powdered plant material were hydrolysed with 2.5% HCl for 2½ hr. at

100° C. over a water-bath, cooled, filtered and the filtrate used for the estimation of carbohydrates.

*Estimation of carbohydrates.*—The method adopted by Doak (1939) as given by McIlroy (1948) was used for clarification of the sugar solution obtained by the above extraction. To 2 ml. of the extracted sugar solution, which was neutralized with KOH, were added 2 ml. of cadmium sulphate solution (26.2 g.  $3\text{ CdSO}_4 \cdot 8\text{H}_2\text{O}$  and 132 ml. N,  $\text{H}_2\text{SO}_4$  made to a litre) and 1 ml. of 0.55 N NaOH. The mixture was heated on a water-bath for three minutes, cooled, filtered and washed. The reducing sugars in the clear filtrate were estimated by the method of Hagedorn and Jensen (1923).

*Total nitrogen.*—Total nitrogen was determined by the micro-Kjeldahl method. Reduction of nitrate was effected by salicylic-sulphuric acid method (A.O.A.C., 1945) using metallic mercury as catalyst. The digested material was distilled in Parna's apparatus and the nitrogen estimated in the usual way.

*Nitrate nitrogen.*—This fraction was calculated by the difference between 'total' and 'total minus nitrate' nitrogen. The 'total minus nitrate' nitrogen was determined just as described above but without reduction of nitrate.

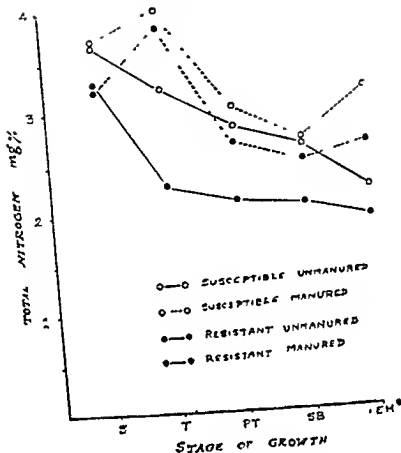
*Protein nitrogen.*—Proteins were precipitated with 5% trichloroacetic acid, ground, filtered and made up to volume with trichloroacetic acid and the nitrogen in aliquots of the filtrate was determined as non-protein (excluding nitrate) nitrogen. Protein nitrogen was calculated as the difference between 'total minus nitrate' nitrogen and non-protein nitrogen.

This fraction of nitrogen, when protein and nitrate nitrogen were deducted from total nitrogen is given as residual nitrogen which included ammoniacal, amino and amide nitrogen.

## EXPERIMENTAL

### 1. Nitrogen Metabolism:

(a) *Total nitrogen* (Text-Fig. 1).—The total nitrogen content of the susceptible type was greater than that of the resistant type in all stages of growth in both the manured and unmanured series. A parallel trend in nitrogen accumulation was seen in both the types. Manuring, in general, increased the total nitrogen content in both the types at all stages of growth, the difference being pronounced at the tillering and earhead stages, and negligible at the seedling stage.

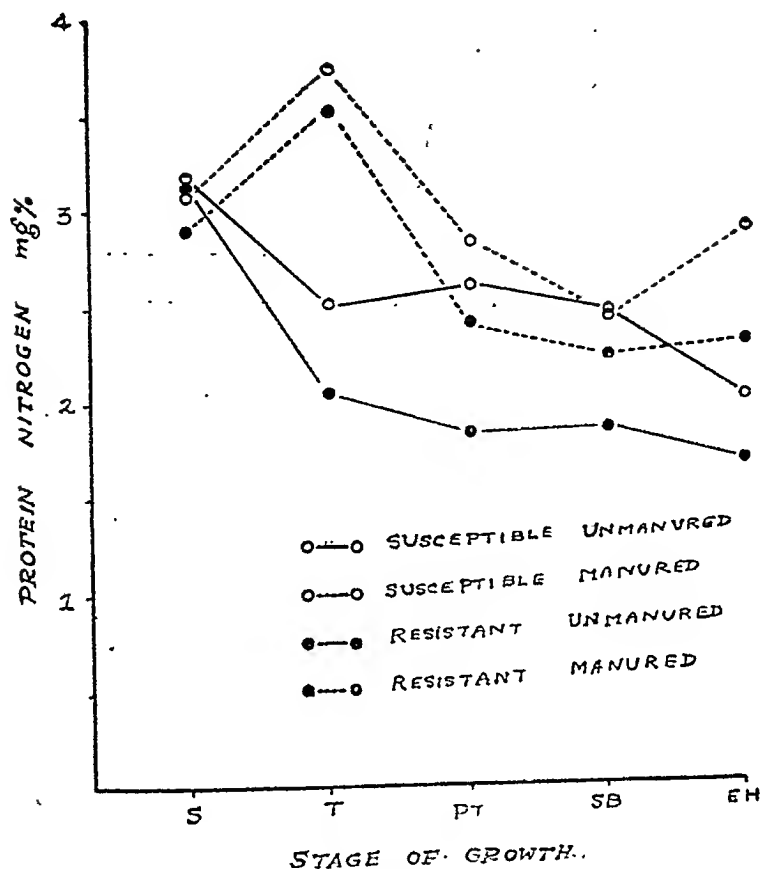


TEXT FIG 1

\* Seedling, T tillering, PT post tillering, SB shot blade and EH earhead stages of rice varieties in all text-figures. For other details, see text

(b) *Protein nitrogen* (Text Fig 2)—Protein nitrogen in both the resistant and susceptible types, with and without manuring, was parallel to that of total nitrogen at all stages of growth. The influence of manuring was again marked at the tillering and the earhead stages, but this influence at the earhead stage was particularly notable in the susceptible type after the shot blade stage when the difference was negligible.

(c) *Nitrate nitrogen* (Text-Fig 3)—Nitrate nitrogen was found in appreciable quantities only up to the tillering stage beyond which its accumulation declined gradually. In the seedling stage the susceptible type showed a higher content of nitrate nitrogen than the resistant type in both the manured and unmanured series.

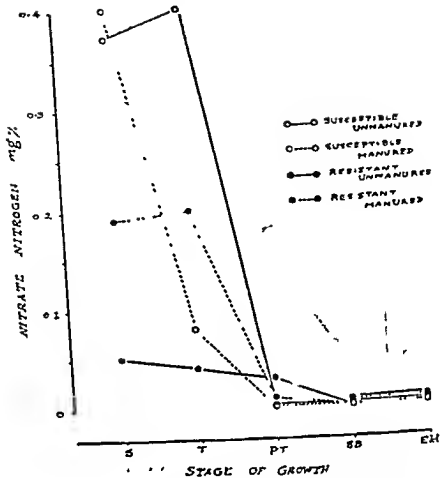


TEXT-FIG. 2

(d) *Residual nitrogen* (Text-Fig. 4).—In general, residual nitrogen in both types was higher in the manured series than in the unmanured except during tillering. In the case of susceptible type the unmanured plants showed a high content of residual nitrogen in the tillering stage. It decreased gradually as the age of the plant increased, with a steep fall in the earhead stage.

## 2. Total Carbohydrates

The distribution of total carbohydrates at different stages of growth of the resistant and susceptible plants subjected to manurial treatments is presented in Text-Fig. 5. In the unmanured series the carbohydrate status was generally the same in both types of plants except in the earhead stage where the resistant types registered a markedly higher carbohydrate content.



TEXT FIG. 3

Manuring decreased the total carbohydrates in both resistant and susceptible plants. This was particularly evident in the tillering stage.

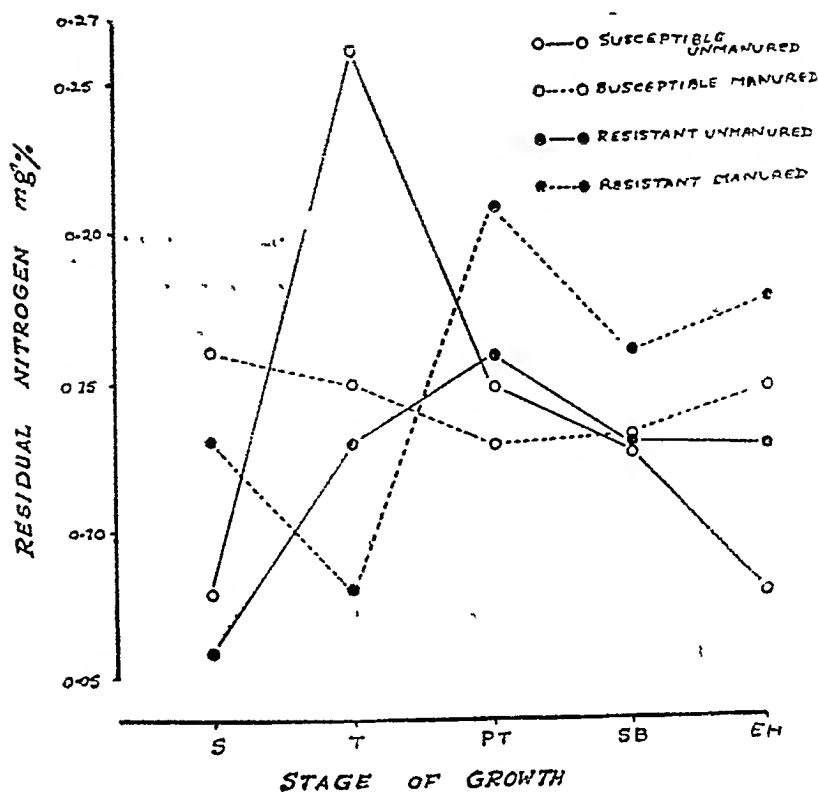
### 3 Carbohydrate/Nitrogen (C/N) Ratio

The C/N ratios for both the types were computed and the results are presented in Text-Fig. 6. The susceptible variety had a lower C/N ratio than the resistant in the unmanured series. Manuring decreased the C/N ratio in both the types.

### DISCUSSION

Some interesting points emerge from the data presented in this paper. Examination of Text-Fig. 1 reveals that a greater quantity of nitrogen is accumulated in the susceptible type than in the resistant throughout the



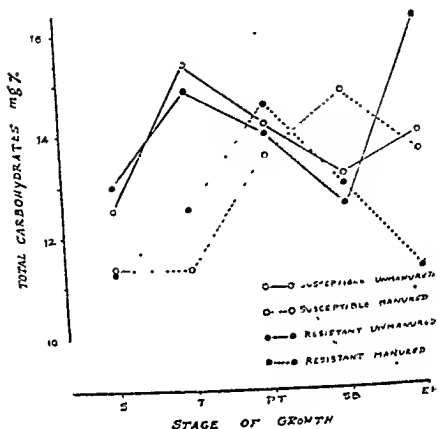


TEXT-FIG. 4

life of the plant. This is in keeping with the observations of Hashiyoka (1944) and Otani (1953) who showed positive correlation between intensity of disease and accumulation of nitrogen in the rice plant. Similarly, Wingard (1941) cited instances of high nitrogen accumulation associated with susceptibility to fungal diseases.

Manuring increased the total nitrogen content of both susceptible and resistant varieties except in seedling stage (Text-Fig. 1). The effect of manuring was best noticed at the tillering and earhead stages. It should be pointed out here that rice plants are most vulnerable to blast at these stages of growth. The lack of response of seedlings to nitrogenous fertilization probably indicates the adequacy of the soil nitrogen itself which is sufficient to meet the demands of the plants at this stage.

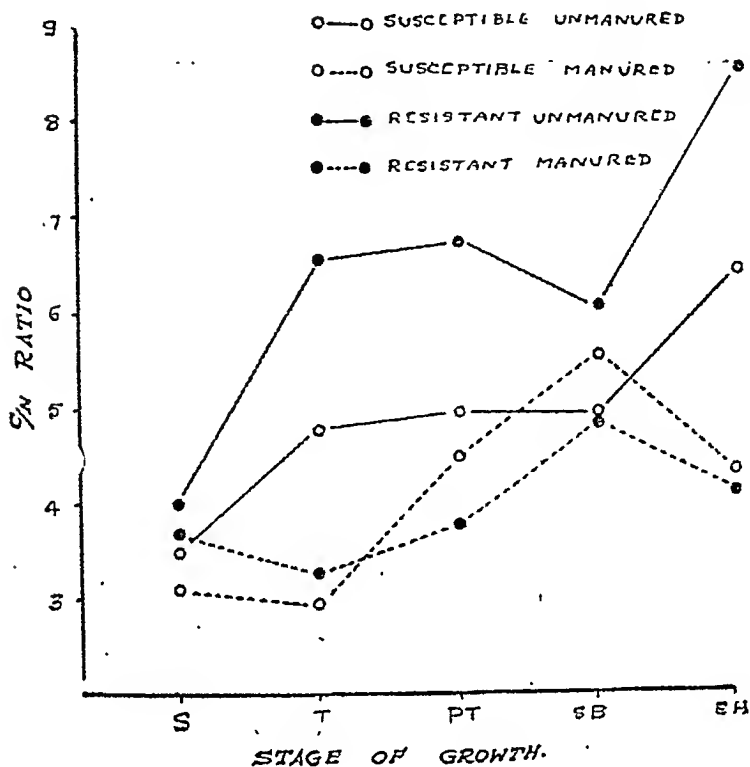
With advancing age, the total nitrogen decreased in both the resistant and susceptible types. It is likely that the nitrogen reserves in the leaves are mobilized for flowering and ear formation (Sen, 1946; Pearsall and



TEXT-FIG. 5

Billimoria, 1937). A similar decline in the nitrogen content during heading of the rice plant was observed by Togasi *et al.* (1954). The gradual fall in the nitrogen content with advancing age however rose with manuring at the earhead stage and this was particularly marked in the case of susceptible type (Text-Fig. 1). Probably under nitrogenous manuring proteolysis in leaves is prevented whereas in the unmanured plants, the nitrogen available in the soil being limited, protein synthesis in the earhead occurs at the expense of leaf nitrogen. Hence, nitrogenous manuring results in an increase of leaf protein and total nitrogen in the susceptible type during the earhead stage when rice plants are known to be most susceptible to neck infection.

Except in the earlier stages of growth, when nitrate was present in appreciable quantities, there was no accumulation of unmetabolized nitrogen in the form of ammonia and nitrate in the rice plant (Text-Fig. 3). Although the figures for ammoniacal nitrogen are not furnished, it was found during



TEXT-FIG. 6

analysis that ammonia was present in negligible quantities. Sircar and Sen (1941) presume that ammonia, if at all present in the rice plant, is negligible.

In general, a decrease in the carbohydrate content was noticed with nitrogenous manuring in both the types (Text-Fig. 5). This is understandable in the light of our knowledge on protein synthesis in plants (Chibnall, 1939; McKee, 1949). The carbon skeletons for protein formation through amino-acids are derived from the organic acids ( $\alpha$ -Keto acids in particular), through carbohydrate dissimilation. As manuring resulted in increased protein synthesis (Text-Fig. 2), the total carbohydrates naturally decreased. This reduction in carbohydrates probably makes the plant more susceptible by impairing cell-wall formation and thus reducing the mechanical resistance of the plant to penetration by the fungus.

In the unmanured series the resistant variety is characterized by a very high C/N ratio. Indeed Hashiyoka (1943 *a*) observed an increase in the

C/N ratio in the rice plant in proportion to rise in temperature and consequently to resistance. However, nitrogenous manuring caused a decrease of C/N ratio in both the types, the ratios being almost identical.

### SUMMARY

A greater accumulation of total and protein nitrogen was noticed in the blast susceptible type of rice plant than the resistant. Nitrogenous manuring increased the total and protein nitrogen content in both the types but still the susceptible types had higher amounts of these than the resistant. The influence of manuring was reflected greatest during the tillering and earhead stages at which periods the rice plant is most susceptible to blast disease. The susceptible variety had a much lower C/N ratio than the resistant in the unmanured series but with nitrogenous manuring the C/N ratio was similar in both types.

### ACKNOWLEDGEMENTS

I am deeply indebted to Prof T S Sadasivan, Director, University Botany Laboratory Madras for suggesting the problem and the keen interest he had taken during the course of this investigation and help in the preparation of this paper. My thanks are also due to Dr C V Subramanian D.Sc F.A.Sc., for many helpful suggestions. To the University of Madras my thanks are due for the award of a scholarship.

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# THE GAMETOPHYTE OF *ACROSTICHUM AUREUM* L.

BY B. K. NAYAR AND (MISS) FARRUKH KAZMI

(Pteridology Laboratory, National Botanic Gardens, Lucknow)

Received October 28, 1963

(Communicated by Dr. V. Puri, F.A.Sc.)

## INTRODUCTION

*Acrostichum* is a pantropic genus of salt-marsh plants, growing in large clusters in coastal areas all over the world. Taxonomically it was one of the most confused genera of ferns: it has long been the practice of pteridologists to associate under it nearly all leptosporangiate ferns with sporangia distributed all over the undersurface of the leaves. Till recently this situation continued, even though the heterogeneous nature of the over-large genus (for example, as conceived by Hooker and Baker, 1868) was long recognised. As now understood, it is a small genus of *ca.* 5 closely similar species (regarded by some pteridologists as only varieties of the type species). The only Indian representative is the type species, *A. aureum* L. (Fig. 33), a salt-marsh fern growing abundantly all over the coastal regions. *Acrostichum* is regarded by most pteridologists (Christensen, 1938; Copeland, 1947; Holttum, 1947; Alston, 1956) as a pteroid fern: among contemporary pteridologists, Ching (1940) treats it as a separate family, *Acrostichaceae*, which, according to him, belongs to the aspidioid phylum. Based on their observations on the gametophyte of one species (*A. speciosum*), Stokey and Atkinson recently concluded that there is "nothing in the gametophyte structure or development which would indicate a close affinity with the Aspidioid ferns, but there are several characters which would ally it with the Pteroid ferns, and, more particularly with the Cheilantheid group" (Stokey and Atkinson, 1952, p. 112).

Though morphologically it is one of the better known genera among the Pteridaceae, our knowledge of the gametophyte of *Acrostichum* is restricted mainly to the report on the prothallus of one species, *A. speciosum* Willd. (Stokey and Atkinson, 1952): a passing reference to some of the prothallial characters of *A. aureum* is made by Schumann (1915) in her detailed morphological study of the sporophyte. Spores for the present study were collected from the West Coast of South India and from the

Andaman Islands, and were cultured on Knop's agar medium using techniques reported in earlier studies (Nayar, 1962*b*). The cultures were maintained throughout at a temperature range of  $24 \pm 2^\circ \text{C}$  and a light intensity of 600 ft c (light supply from Phillips' fluorescent lamps, for 12 hr duration in every 24 hr period). Spore morphological observations are based on acetolysed preparations mounted in glycerine jelly (Erdtman, 1952). Spore measurements are averages of 20 readings in each plane.

### Spore

The spores of *A. aureum* are trilete (tetrahedral), with a nearly triangular amb having smoothly rounded broad corners and faintly concave sides (Fig. 1). The laesura is tenuimarginate and each arm is ca  $21 \mu$  long. The spores measure  $53 \times 60 \mu$  (P  $\times$  E). The exine is ca  $4.0 \mu$  thick and clearly demarcated into a sexine and nexine; the sexine is conspicuously thicker than the nexine and is densely granulose. Fresh spores contain dense, nearly colourless plastids and one or two pale-yellowish oil globules.

### Prothallus

In about 4–6 days of sowing, the spores turn pale green and germinate by the exine splitting open at the laesura and the first rhizoid emerging as a hyaline protrusion. Chloroplasts are not found included in the rhizoid, but the prothallial cell, which soon elongates as the germ filament, is densely chlorophyllous. The germ filament (Fig. 2) becomes 6–8 cells long in about a week after spore germination. The cells of the germ filament are generally short and broader than long. The basal cell is slightly swollen and enveloped by the spore coat which remains attached to it for long. Generally the germ filaments are more or less tapering towards the apex, the anterior cells being progressively narrower (Figs 2–3). Growth of the germ filament is mainly by repeated divisions in the intercalary cells, and, as reported in *A. spectosum* (Stokey and Atkinson, 1952), there is little elongation of cells during the development of the germ filament and the intercalary cells often appear disk-like. Prominent, yellowish-brown oil globules are present in the basal cell, and in most cases these coalesce to form a large, irregularly shaped mass occupying nearly half the volume of the cell and persisting till a distinct prothallial plate is developed. As the germ filament becomes 6–8 cells long the anterior cells appear to become more or less quiescent. The terminal cell soon stops growth and may become more or less conical.

Formation of a prothallial plate is initiated by longitudinal divisions in the intercalary cells, generally two or three cells behind the terminal cell.

(Fig. 3). Most of the cells, except often two or three at either end of the germ filament, now divide transversely again and expand laterally, becoming dorsiventrally flattened (Fig. 4). Soon each intercalary cell divides by a median longitudinal wall and the prothallus is then nearly ribbon-like (Fig. 5). The terminal cell of the germ filament scarcely expands, but, due to the lateral expansion of the penultimate cell, appears nearly conical or even more or less beaked (Fig. 4). The penultimate cell often divides longitudinally soon and take part in the formation of an expanded prothallial plate (Figs. 4, 5), but the terminal cell usually remains quiescent, though rarely it may divide unequally once (Fig. 9). In many cases, two or three of the cells in the terminal region remains uniseriate, forming a small beak on the expanding prothallial plate (Figs. 12-14). In no case an obconic meristematic cell is developed from the terminal cell as reported by Schumann (1915). In one case the terminal cell of a germ filament was observed, in which two oblique divisions cut off an obconical daughter cell (Fig. 8): however, this cell was not meristematic. The basal cell of the germ filament often develops a cluster of rhizoids similar to the first—nearly hyaline, non-chlorophyllous and with a slightly swollen base. Rhizoids are rarely developed by the other cells of the prothallus till the anterior region of the prothallus expands and becomes three or more cells broad. In the posterior half, all, except the basal cell, often divide longitudinally and take part in plate formation (Fig. 9). Branching of the germ filament is rare: but in some cases as the terminal region of the germ filament becomes gradually quiescent, a lateral branch may be developed by one of the daughter cells formed by longitudinal division of an intercalary cell. This branch then continues the growth of the prothallus (Figs. 10, 11). Rarely a group of intercalary cells on one side develops into a broad lateral lobe: this lobe expands and develops into the prothallus while the uniseriate anterior region remains as a beak-like protuberance (Fig. 12). Young prothalli of *A. aureum* are characteristic in being broad and nearly ribbon-like, often with the anterior as well as the posterior ends more or less tapering and the intercalary region composed of many, narrow, transversely elongated, disk-like cells (Figs. 5-9).

The prothallus expands, and, in about 2 weeks after sowing the spores, becomes nearly spatulate. Lateral cells on one side, nearly towards or a little below the middle of the spatulate region (Fig. 7), now divide more frequently than the others: this region later grows out as a broad lobe (Fig. 13). The terminal cell of the prothallus (which remains uniseriate), in some cases produces an apical rhizoid (Fig. 16, *r*) and other rhizoids are developed by some of the basal cells. The lateral lobe expands considerably and consequently the terminal region of the prothallial plate is pushed to one side

*A. speciosum* (Stokey and Atkinson, 1952), but the neck is comparatively shorter, being only 4 or 5 cells long (Fig 24). The neck canal cell is usually binucleate, but, as in *A. speciosum*, was sometimes found to contain four nuclei. Three nucleate neck canal cells, however, were not observed in *A. aureum*. The development of the archegonium is of the common type (Figs 21-23). Archegonial initials are differentiated generally 5-7 cells behind the meristem of the prothallus (Fig 21). During development, the neck canal nucleus divides prior to the differentiation of a ventral canal nucleus (Fig. 23). As the archegonia attain maturity the prothallial cells surrounding the egg divide and form a well differentiated venter composed of a row of regularly arranged small cells (Fig 24).

#### JUVENILE SPOROPHYTE

The early juvenile leaves of the sporophyte are generally spatulate and nearly entire (Figs 26-29). The simplest ones may be broadly obtuse with a rounded apex. The single vein supplying the lamina is forked once or twice. Generally the very first juvenile leaf is spatulate with the vein dichotomising two or three times (Fig 31). Two or three of the succeeding leaves may be similar but larger. Often a large central areole, bearing excurrent veinlets, may be formed by fusion of some of the ultimate veinlets (Fig 29). A midrib is developed by the third or the fourth leaf, and soon areoles are developed on either side of it (Fig 30). The apex of the lamina becomes pronounced. A distinctly oblong lamina with a central midrib and profusely reticulate venation resembling the adult pattern is developed by the eighth to the tenth leaf (Fig 32). All the juvenile leaves possess a simple lamina with a nearly entire margin. Though herbaceous, the lamina is thick, in marked contrast to the thin membranous juvenile lamina of most of the ferns (see Fig 34). The early juvenile leaves are naked. Multicellular hairs and paleae are developed at the stipe base. Multicellular hairs with slightly swollen terminal cells occur sparsely over the stipe of the third juvenile leaf onwards. Similar but smaller hairs gradually spread over the main veins (but are sparse) in later-formed leaves.

#### DISCUSSION

The prothallus of *A. aureum* is closely similar to that of *A. speciosum* (Stokey and Atkinson, 1952), in its morphology and development, even though there are minor differences especially with regard to the development of a prothallial plate. In *A. aureum* the prothallial plate expands laterally by the increased activity of some of the lateral intercalary cells of the ribbon-like thallus and develops into a broad spatulate plate in which a

meristem is differentiated on the side facing the posterior end of the prothallus, contrary to the condition in *A. speciosum*. The major features which characterise the prothallus of *Acrostichum* are: the intercalary growth of the germ filament by the addition of more cells rather than by cell elongation, the completely lateral development of the meristem, the omission of an apical-cell-stage in development, the nature and behaviour of the terminal cell of the germ filament, the asymmetric nature of the mature prothallus, lack of hairs on the prothallus, and the beaked nature of the opercular cell of the antheridium. A lateral development of the meristem is found in several genera of the Pteridaceae, including *Pteris*, but is not a universal feature in the family: it is more conspicuous in *Pityrogramma* and *Onychium*, but in these genera the terminal region of the germ filament is not quiescent as in *Acrostichum*. A condition similar to that in *Onychium* etc., is reported in some schizaeoid ferns (Atkinson, 1960; Kaur, 1961). Among the aspidioid ferns (from which group Ching derives the Acrostichaceae) the meristem usually is lateral in the early stages of development, but this initial asymmetric development, as pointed out by Stokey and Atkinson (1952), does not appear to be comparable to the distinctly lopsided development of *Acrostichum*. In many of the aspidioid ferns, the terminal cell of the germ filament stops growth and ends in a hair: a meristematic cell is then developed by an oblique division in one of the penultimate cells (Nayar, 1960 *a*; Nayar and Kaur, 1963; Nayar and Chandra, 1963). The beaked nature of the quiescent terminal cell of *Acrostichum* does not appear to be comparable to this terminal hair of the Aspidioid ferns. Moreover, the prothalli of all aspidioid ferns, as far as are known, are profusely hairy in contrast to the naked thalli of *Acrostichum*. Also, the spores of the Aspidiaceae are of the monolete type, enveloped by a distinct perine (Nayar and Devi, 1963, 1964). The trilete spores of *Acrostichum* ally it to the Pteridaceae, a family characterised by trilete spores. However, the spores of the pteroid ferns, *Onychium*, *Pityrogramma*, *Actinopteris* (Nayar, 1962 *c*), etc., possess a characteristic equatorial ridge.

The juvenile leaves of *Acrostichum* also differ from the usual type in the Pteridaceae in possessing a simple, entire lamina: a lobed or dissected lamina characterise the Pteridaceae (Nayar, 1962 *c*, 1962 *d*, 1962 *e*; Nayar and Kaur, 1963). The early juvenile leaves of some of the cheilantheid ferns (Nayar, 1956, 1960 *b*, 1962 *a*, 1963; Nayar and Bajpai, 1964) tend to be less dissected to nearly entire, but they soon become lobed as a midrib is formed, and are profusely hairy.

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## SUMMARY

The spores of *A. aureum* are trilete and granulose. On germination, a 6-10 cells long germ filament is produced, in which growth is mainly by intercalary formation of new cells rather than by cell elongation. One or two of the terminal cells become quiescent soon, and the intercalary cells form a prothallial plate. Cells on one side of the plate are more active than those on the other and a broad ameristic lateral lobe is developed by their activity. As the lobe becomes spatulate, a multicellular meristem is differentiated from marginal cells on the side facing the posterior end of the germ filament. By the activity of the meristem the prothallus becomes cordate, with the meristem at the bottom of the notch. A midrib is formed behind the notch and the prothallus grows to become asymmetrically cordate. The mature prothallus is naked and with ribbed wings. Juvenile leaves possess entire, naked lamina.

## ACKNOWLEDGEMENTS

It is a pleasure for us to record here our gratitude to Prof K. N. Kaul, Director, National Botanic Gardens, for the keen interest he has evinced in this work. We are thankful to Mr Lalit Prasad Tiwari for help in microtomy and staining.

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### EXPLANATION OF FIGURES

FIGS. 1-32. Fig. 1. Proximal view of spore. Fig. 2. A 5-day-old germ filament. Fig. 3. Germ filament, showing initiation of plate formation by longitudinal division in an intercalary cell. Figs. 4-6. Formation of a prothallial plate. Fig. 7. A 2-week-old prothallus, showing increased meristematic activity of lateral cells on one side. Figs. 8, 9. Young prothalli, showing divided terminal cell. Figs. 10, 11. Development of a lateral lobe in young prothalli. Fig. 12. Young prothallus, showing an enlarged lateral lobe and quiescent terminal region. Figs. 13-15. Stages in the development of a lopsided prothallial plate (x, meristematic region, t, terminal region). Fig. 16. A 3-week-old prothallus, showing development of a notched 'apex' (x), and the terminal region of the prothallus (t) ending in a rhizoid (r). Fig. 17.

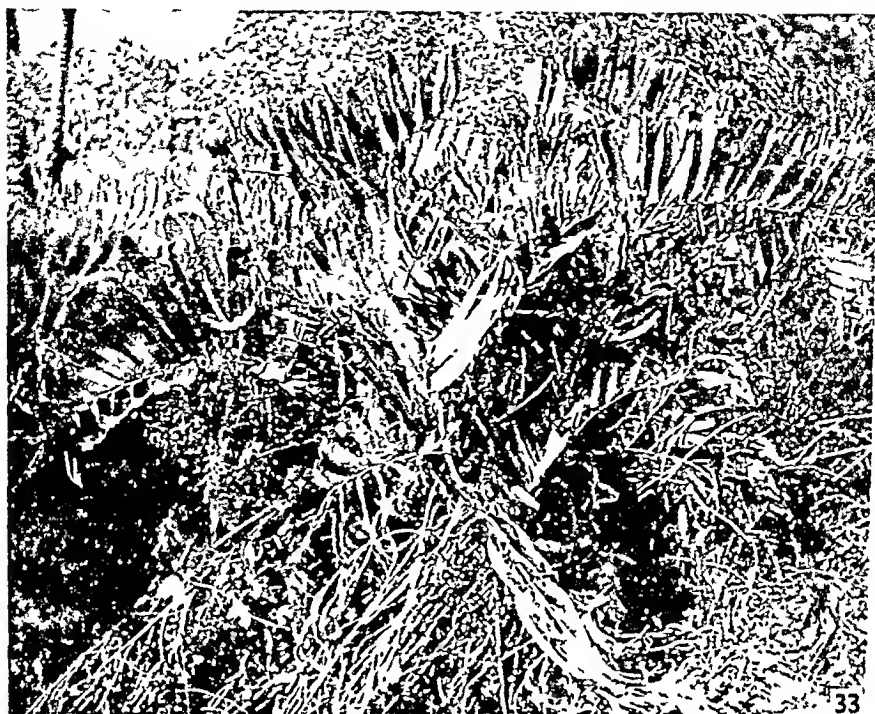
Mature prothallus (♀, archegonium) Fig. 18 Mature antheridium. Fig. 19 Antheridium, showing dehiscence (o, pore like opening) Fig. 20 Stalked antheridium Fig. 21 Ls of apical region of mature prothallus, showing initiation of archegonium (♀, archegonium) Figs. 22, 23 Stages in development of young archegonium Fig. 24 Mature archegonium Fig. 25 Apical region of mature prothallus (♀, archegonium) Figs 26-32. Juvenile leaves.

### EXPLANATION OF PLATE VIII

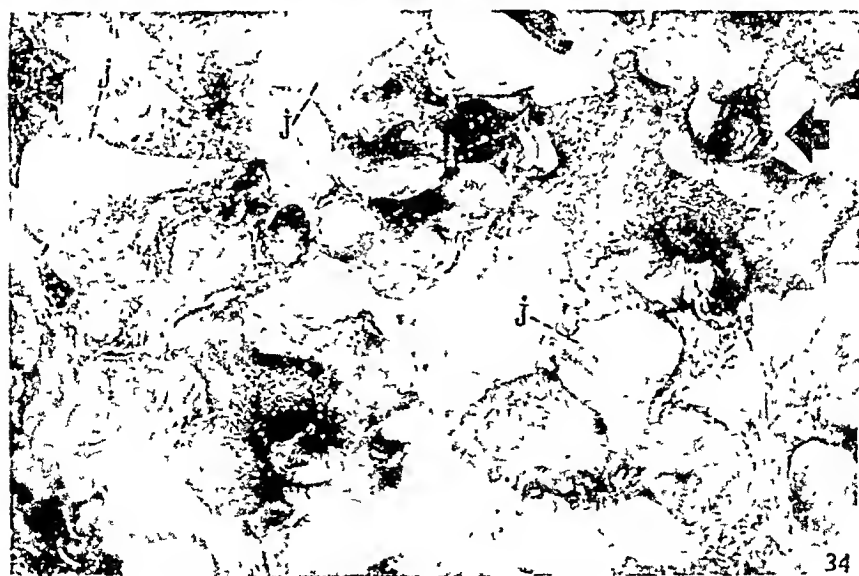
FIG 33 *Aerostichum aureum* growing in a marshy area on the Kerala coast.

FIG 34 Cultured prothalli of *A. aureum*

(J, juvenile plants. The arrow on the right points to the uplifted terminal region of a prothallus.)



33



34

FIGS. 33-34

# THE BLOOD VASCULAR SYSTEM OF *RIOPA GUENTHERI* (PETERS) REPTILIA: SAURIA

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## INTRODUCTION

*Riopa guentheri* belongs to the family Scincidae and is fairly common at Castle-rock, a place in the Western Ghats, about 60 miles south-west of Dharwar, Mysore State, India. They are cryptic and lie hidden underneath stones. They seem to prefer hill tops. Castle-rock enjoys a good rainfall averaging about 90 inches per year. The lizard is peculiar in possessing very rudimentary limbs and exhibiting a sort of fossorial life. The present work gives a general morphological account of the heart and the vascular system and also examines whether the reduction in the limbs and the lizard's peculiar mode of life have any bearing on the vascular system.

## MATERIAL AND METHOD

The specimens were kept alive in the laboratory till such time as they were used for dissection. As the lizard is small, the blood-vessels are very slender and therefore injection methods were not a success. However, Indian ink injection through the heart rendered the arteries darker and facilitated their dissection to some extent. The venous system had to be studied in freshly killed specimens.

## THE HEART

A detailed account of the heart of *Riopa guentheri* has already been given in a paper published by one of us (Kashyap, 1951) and, therefore, only a general description is given here. The heart lies in the pericardial cavity, slightly in front of the line joining the two axilla. The anterior position of the heart is, according to Rathke (1848), an indication of a lower grade of organisation. The heart is only slightly elongated and possesses a gubernaculum cordis connecting the ventricular apex with the pericardium. The

sinus venosus (Fig 2, *sin ven*) is not sharply marked off from the base of the three venous trunks. The sinu-auricular aperture is elliptical in shape and obliquely transverse in position. It is guarded by well-developed anterior and posterior valves. The right auricle (Figs 1, 2 and 4, *rt aur*), as in most reptiles, is larger than the left (Figs 1, 2 and 4, *lt aur*) and bears a rounded diverticulum (Fig 2, *divert*) in its anterior portion, towards the median line. The pulmonary vein is single and reaches the left auricle from the posterior side (Fig 2, *pul vein*). The auriculo-ventricular apertures are guarded, each, by a single large mesial valve attached to the posterior portion of the inter auricular septum. It is also attached to the wall of the ventricle along its dorsal and ventral margins. Its lateral margin is free, indented and inflexed. There are no chordae tendinae. The ventricle (Figs 1, 2 and 4, *vent*) has two cavities, a large left-dorsal cavum dorsale and a small right ventral cavum pulmonale. The two cavities are separated by an incomplete interventricular septum.

### THE ARTERIAL SYSTEM

The three aortic arches arise in the same antero-posterior sequence as they do in the heart of other lizards, namely, right systemic, left systemic

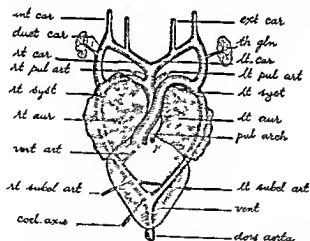


FIG 1 *Riopa guentheri*—Ventral view of the heart showing the aortic arches and their branches.

*coel axis*, coeliac axis, *dors aorta*, dorsal aorta, *duct car*, ductus caroticus, *ext car*, external carotid, *int car*, internal carotid, *lt aur*, left auricle, *lt car*, left carotid, *lt pul art*, left pulmonary artery, *lt subcl art*, left subclavian artery, *lt syst*, left systemic arch, *pul arch*, pulmonary arch, *rt aur*, right auricle, *rt car*, right carotid, *rt pul art*, right pulmonary artery, *rt subcl art*, right subclavian artery, *rt syst*, right systemic arch, *th gln*, thyroid gland, *vent*, ventricle; *vent art*, vertebral artery

and the pulmonary arch. The three arches seem to arise from a small swelling situated near the base of the ventricle, slightly to the right of the median line on the ventral aspect. O'Donoghue (1920) reports a similar swelling in the heart of *Sphenodon punctatus* and regards it as a remnant of the conus arteriosus. Such a swelling is common in the hearts of lizards and snakes. Soon after their origin, the aortic arches undergo a slight twist so that the pulmonary arch which is towards the left of the left systemic arch at the point of origin, shifts to a position dorsal to both the left and right systemic arches. The left systemic arch arises from the right side of the base of the ventricle while the right systemic arch arises from the left side. This condition is common to most of the reptiles. The coiling of the aortic arches, however, may be loose (Chelonia) or tight (Lacertilia and Ophidia).

The pulmonary arch (Fig. 1, *pul. arch*) is a large trunk which arises from the ventral portion of the base of the ventricle, slightly to the right side, as an anterior continuation of the cavum pulmonale. It soon bifurcates into a pair of pulmonary arteries (Figs. 1 and 2, *lt. pul. art.*; *rt. pul. art.*) which take the blood to the two lungs. As in *Sphenodon punctatus* (O'Donoghue, 1920), *Calotes versicolor* (Das and Mitter, 1928) and *Hemidactylus flaviviridis* (Bhatia and Dayal, 1933), in *Riopa guentheri* also there is a tracheal artery arising from each pulmonary artery (Fig. 2, *lt. tr. art.*; *rt. tr. art.*). The tracheal artery runs anteriorly and ends in a capillary network on the trachea. This is also considered to be a primitive feature and has been described in *Sphenodon punctatus* by O'Donoghue (1920).

The left systemic arch (Figs. 1, 2 and 4, *lt. syst.*) emerges from the ventricle, slightly ventral to the right systemic arch, runs obliquely for a distance and then posteriorly to meet the right systemic arch to form the dorsal aorta. The opening of the left systemic arch is situated near the anterior end of the incomplete interventricular septum, slightly dorsal to it and adjacent to the opening of the right systemic arch. The left systemic arch does not bear any branch except the ductus caroticus of its side (Fig. 1, *duct. car.*).

The presence of a ductus caroticus, a connection between the carotid trunk and the systemic arch, has also been reported in *Calotes versicolor* (Das and Mitter, 1928), *Hemidactylus flaviviridis* (Bhatia and Dayal, 1933; Mahendra, 1942) and *Gecko verticillatus* (Das and Das, 1931). The ductus caroticus in *Riopa guentheri* presents a striking appearance being so big as to seem that it is the main vessel and the carotid trunk a small branch arising from it. This fact seems to emphasise the primitive nature of the lizard, for, with the ductus caroticus so large, it is likely that the blood contained



in the left carotid trunk and the left systemic arch mix freely. The ductus caroticus gives off a small branch called the arteria muscularis cervicis to the muscles of the neck and the thymus gland. Such a branch has been observed in *Gecko verticillatus* (Das and Mitter, 1928) and *Hemidactylus flaviviridis* (Bhatia and Dayal, 1933, Mahendra, 1942).

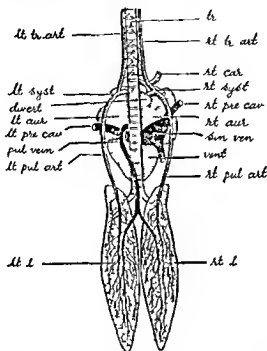


FIG. 2. *Ruopa guentheri*.—Dorsal view of the heart showing the pulmonary arch, the tracheal artery and the pulmonary vein.

*divert*, diverticulum, *lt aur*, left auricle, *lt l*, left lung, *lt pre cav*, left precaval, *lt pul art*, left pulmonary artery, *lt syst*, left systemic arch, *lt tr art*, left tracheal artery, *pul vein*, pulmonary vein, *rt aur*, right auricle, *rt car*, right carotid, *rt l*, right lung, *rt pre cav*, right precaval, *rt pul art*, right pulmonary artery, *rt syst*, right systemic arch, *rt tr art*, right tracheal artery, *tr*, trachea, *sin ven*, sinus venosus, *vent*, ventricle.

The right systemic arch (Figs 1, 2 and 4, *rt syst*) emerges dorsal to both the pulmonary and the left systemic arches. Its opening is situated slightly mesial and anterior to that of the left systemic at the right basal corner of the *cavum dorsale*. In a level with the anterior margin of the auricles, the right systemic arch gives off a common carotid trunk, gradually curves round the heart and then runs posteriorly to meet the left systemic arch. The junction of the two systemic arches, as in most reptiles excepting the chelonians, is dorsal to the heart, in a level with the middle of the ventricle.

VESSELS ARISING FROM THE RIGHT SYSTEMIC ARCH

(a) The common carotid trunk arises from the right systemic arch at about the level of the anterior margin of the auricles and very soon divides into left and right branches (Figs. 1, 2 and 4, *lt. car.* ; *rt. car.*). Each branch gives off an internal and an external carotid artery (Fig. 1, *int. car.* ; *ext. car.*). The internal carotid artery runs lateral to the brain and divides into two branches. One of these passes beneath the columella, enters the snout region and breaks up into smaller branches. The other branch runs alongside the brain and breaks up into finer vessels in the anterior region. The external carotid artery gives off branches to the hyoid apparatus and the tongue.

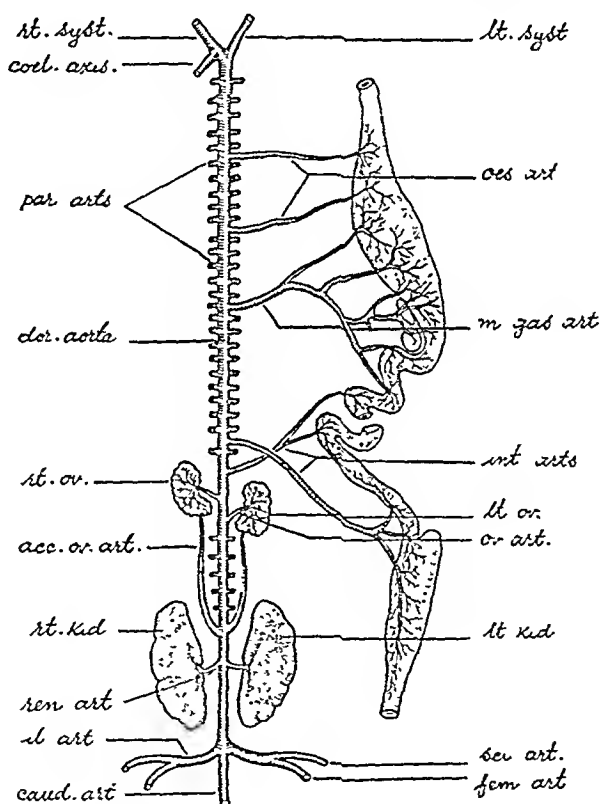


FIG. 3. *Riopa guentheri*—The dorsal aorta and its branches.

*acc. ov. art.*, accessory ovarian artery; *caud. art.*, caudal artery; *coel. axis*, coeliac axis; *dors. aorta*, dorsal aorta; *fem. art.*, femoral artery; *il. art.*, iliac artery; *int. arts.*, intestinal arteries; *lt. kid.*, left kidney; *lt. ov.*, left ovary; *lt. syst.*, left systemic arch; *m. gas. art.*, mid-gastric artery; *oes. art.*, oesophageal artery; *ov. art.*, ovarian artery; *par. arts.*, parietal arteries; *ren. art.*, renal artery; *rt. kid.*, right kidney; *rt. ov.*, right ovary; *rt. syst.*, right systemic arch; *sci. art.*, sciatic artery.

(b) The vertebral artery (Fig 1, *vert art*) is the first vessel to arise from the right systemic arch after it has given off the common carotid trunk. It runs by the side of the vertebral column and supplies blood to it.

(c) Next to the vertebral artery, further posteriorly, arise the left and right subclavian arteries (Fig 1, *lt subcl art*, *rt subcl art*). There appears to be a number of variations regarding the origin of the two subclavian arteries among the lizards that have been studied. In *Lacerta viridis* (Saunders and Manton, 1931) the two subclavians originate at the same level, opposite each other, while in *Varanus* they arise as a single branch and then bifurcate into left and right subclavian arteries. Each one of them gives off a vertebral artery. In *Riopa guentheri* the two subclavians arise at different levels, the right one being anterior to the left. As already referred to, the vertebral artery has an independent origin.

(d) At the junction of the two systemic trunks arises a small vessel called the coeliac axis (Fig 1, *coel axis*) which supplies blood to the muscles of the body wall.

### THE DORSAL AORTA

The dorsal aorta (Figs 1 and 3, *dor aorta*) is the biggest arterial trunk and is formed by the fusion of the two systemic arches and runs along the whole length of the body beneath the vertebral column. Along its length it gives off a series of paired lateral branches called the parietal arteries (Fig 3, *par arts*) which supply blood to the muscles and the body wall. There are about 25-35 pairs of these segmentally arranged vessels. The dorsal aorta also supplies blood to the various organs situated in the abdominal cavity by means of several branches which have been described below in their antero-posterior sequence.

(a) Two oesophageal arteries (Fig 3, *oes arts*) arise about the level of the fifth and tenth pair of parietal arteries and supply blood to the oesophagus.

(b) The mid-gastric artery (Fig 3, *m gas art*) arises next in order, at about the level of the fifteenth pair of parietal arteries and soon bifurcates into two branches. The anterior branch supplies the stomach while the posterior one supplies not only the posterior part of the stomach but also the duodenum, pancreas and spleen.

(c) The intestinal arteries (Fig 3, *int arts*) are two in number and arise at about the level of the twenty-eighth or thirtieth pair of parietal arteries. The anterior intestinal artery runs across the posterior one and supplies blood

to the lower parts of the alimentary canal. The posterior intestinal artery supplies the small intestine.

(d) The gonadal arteries are paired and arise posterior to the intestinal arteries and supply blood to the gonads. The gonads are also supplied with blood by a pair of accessory gonadal arteries arising more posteriorly near the kidneys (Fig. 3, *ov. art.*; *acc. ov. art.*).

(e) The renal arteries form a pair of vessels arising opposite to each other in a level with the middle portion of the kidney and supplying blood to that organ (Fig. 3, *ren. art.*).

(f) Finally, the dorsal aorta gives off a branch to each of the hind limbs and continues posteriorly as the caudal artery (Fig. 3, *caud. art.*). The branch supplying blood to the hind limb is called the iliac artery (Fig. 3, *il. art.*) and it divides soon into the sciatic and femoral arteries (Fig. 3, *sci. art.*; *fem. art.*).

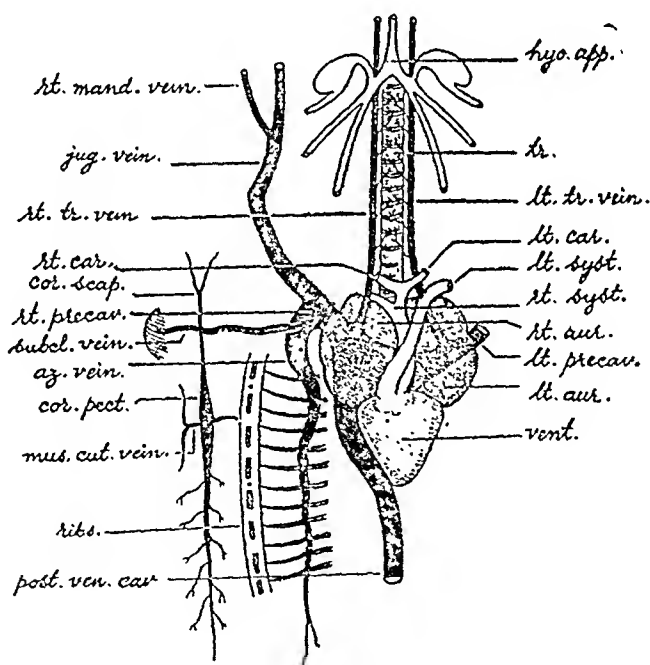


FIG. 4. *Riopa guentheri*—The anterior veins.

*az. vein*, azygos vein; *cor. pect.*, coraco-pectoralis vein; *cor. scap.*, coraco-scapularis vein; *hyo. app.*, hyoid apparatus; *jug. vein*, jugular vein; *lt. aur.*, left auricle; *lt. car.*, left carotid; *lt. pre. cav.*, left precaval; *lt. syst.*, left systemic arch; *lt. tr. vein.*, left tracheal vein; *mus. cut. vein.*, musculo-cutaneous vein; *post. ven. cav.*, posterior vena cava; *rt. aur.*, right auricle; *rt. car.*, right carotid; *rt. mand. vein.*, right mandibular vein; *rt. pre. cav.*, right precaval; *rt. syst.*, right systemic arch; *rt. tr. vein.*, right tracheal vein; *subcl. vein*, subclavian vein; *tr.*, trachea; *vent.*, ventricle.

## THE VENOUS SYSTEM

The venous system of *Riopa guentheri* resembles that of *Hemidactylus flaviviridis* (Mahendra, 1942) in its main features. For the sake of description the venous system has been divided into anterior and posterior divisions.

*The anterior venous system*

The venous system of the anterior region shows certain peculiarities which signify a primitive condition. Blood from the anterior region is drained into the sinus venosus by the right and left anterior venae cavae. Both being similar in disposition, only the right anterior vena cava has been described in detail.

The right anterior vena cava or the right precaval (Figs 2 and 4, *rt pre cav*) is formed by three principal vessels, namely, the tracheal, the common jugular and the subclavian. The mode of union of these three vessels vary slightly in different lizards. In *Hemidactylus flaviviridis* (Mahendra, 1942) the tracheal vein joins the common jugular vein quite anteriorly while the subclavian does so at its root. Mahendra (1942) refers to the fact that in *Psammisaurus griseus* (Corti, 1853) and *Varanus bengalensis* (Thapar, 1921) the anterior vena cava is formed by two veins only, namely, the jugular and the subclavian. In the rhynchocephalian *Sphenodon punctatus* (O'Donoghue, 1920) the three trunks meet at a point. In *Riopa guentheri*, however, a strikingly different disposition is seen. The tracheal vein (Fig 4, *tr vein*, *rt tr vein*) opens into the base of the precaval while the subclavian vein (Fig 4, *subcl vein*) opens into the base of the large azygos vein (Fig 4, *az vein*) instead of opening into the jugular vein (Fig 4, *jug vein*).

The tracheal vein is a fairly large vessel which runs dorsal and parallel to the comparatively smaller tracheal artery. The opening of the tracheal vein into the base of the precaval is hidden from view by the anterior portions of the auricle. As in *Sphenodon punctatus* (O'Donoghue, 1920) and *Hemidactylus flaviviridis* (Mahendra, 1942) the right tracheal vein is longer than the left. Anteriorly the tracheal vein receives the lingual vein and, in the snout region, small tributaries from muscles.

*Riopa guentheri* resembles *Hemidactylus flaviviridis* (Mahendra, 1942) in the absence of the anastomosis between the two tracheal veins. This condition is presumed to be a primitive one. Even in *Sphenodon punctatus* (O'Donoghue, 1920) there is a sort of a network between the two tracheal veins and in *Varanus bengalensis* (Thapar, 1921) there are four anastomoses at different points.

The jugular vein is a stout vessel which receives a single branch, namely, the mandibular vein (Fig. 4, *rt. mand. vein*). It drains the blood from the neck and the adjacent muscles with the aid of minute tributaries. The mandibular vein joins the jugular vein near the angle of the jaw. The jugular vein and the mandibular vein thus collect blood from different parts of the head anterior to the neck. The external jugular vein may be taken to be wanting altogether or may be supposed to be represented by some insignificant vessel joining the jugular vein.

The subclavian vein (Fig. 4, *subcl. vein*) is a very thin vessel which pursues a straight course from the base of the limb to the base of the precaval. It fetches blood from the forelimb, shoulder girdle and the body wall. The vessels taking part in the formation of the subclavian are the same as in *Hemidactylus flaviviridis* (Mahendra, 1942) and *Sphenodon punctatus* (O'Donoghue, 1920) and they are vena coraco-scapularis, vena musculo-cutaneous and vena coraco-pectoralis (Fig. 4, *cor. scap.*; *musc. cut. vein*; *cor. pect.*). Of these, the last-named is fairly large and may be described in some detail. In its size, the coraco-pectoralis appears to be stouter than the subclavian of which it is a tributary. It collects blood from the lateral part of the thoracic region. It is thin at either ends and somewhat dilated in the middle. Along its course, it receives segmentally arranged vessels which run parallel to the spinal nerves. The coraco-pectoralis also receives a small branch opposite to the point where the musculo-cutaneous vein joins it. This small branch brings blood from the intercostal muscles and does not seem to be represented in other lizards.

The azygos vein (Fig. 4, *az. vein*) collects blood from the costal and vertebral region and drains it into the precaval. It is very much larger than the subclavian vein which opens into it. It may be noted that, probably as a result of the reduction of the forelimb, the subclavian vein has undergone a corresponding reduction in size. The fossorial mode of living as also the active habits of the lizard may have given a greater emphasis to the azygos vein which drains the blood from the thoracic region which is concerned with respiratory movements.

#### *The Posterior Venous System*

Blood from the posterior region is drained into the heart by the large posterior vena cava (Figs. 4 and 5, *post. ven. cav.*). It is formed by the union of the two venae renales revehentes (Fig. 5, *lt. ren. reve.*; *rt. ren. reve.*). The union occurs at about the level of the gonads. The posterior vena cava runs through the substance of the right liver lobe, entering its posterior prolonga-

tion dorsally Inside the liver it runs quite superficially and leaves it at its anterior margin, ventrally About eight to ten more or less prominent vessels join the posterior vena cava during its passage through the liver These vessels bring blood from the body wall After emerging from the liver, the posterior vena cava runs straight towards the heart and opens into the sinus venosus

The venae renales revehentes are paired in *Riopa guentheri* They transport blood from the kidneys and hence act as efferent renal vessels Mahendra (1942) while referring to these veins in *Hemidactylus flaviviridis* classifies the venae renales revehentes met with among the lizards into three types, on the basis of their origin The first type arises posterior to the kidneys as a single vessel, bifurcates into two branches which run anteriorly and fuse again to form the posterior vena cava This type is found in *Pygopus lepidopus* (Beddard, 1904) and *Varamus bengalensis* (Thapar, 1921, Bhattacharya 1921) The second type arises as paired vessels in between the two kidneys interconnected, in some by transverse vessels This type is found in *Lacerta* (Hochstetter, 1893) and *Hemidactylus flaviviridis* (Mahendra, 1942) The third type also arises in between the two kidneys rather in the form of a large sinus which receives numerous vessels from the kidneys This type has been reported by Bhatia (1929) in *Uromastix hardwickii* In *Riopa guentheri* it is of the inter renal type similar to the one found in *Hemidactylus flaviviridis* (Mahendra, 1942) The two venae renales revehentes are interconnected, towards their posterior end, by three short transverse vessels referred to as the inter renal anastomoses (Fig 5, *int ren anast*) The number and position of such transverse vessels are variable In *Sphenodon punctatus* (O'Donoghue, 1920) there are four transverse vessels, in *Lacerta viridis* (Saunders and Manton, 1931) one and in *Hemidactylus flaviviridis* (Mahendra, 1942) two In these the transverse vessels are not restricted to the posterior portions of the venae renales revehentes

As is commonly seen in other lizards, in *Riopa guentheri* also the right vena renales revehentes is slightly shorter than the left Their diameters are, however, equal and they contribute equally to the formation of the posterior vena cava In this respect it resembles only one other lizard, *Varamus bengalensis* (Thapar, 1921) The gonadal and renal veins open into the venae renales revehentes

In *Riopa guentheri* the spermatic veins are more than one in number The spermatic veins of the left side open into the vena renales revehentes of their side while those of the right side open directly into the postcaval This condition is similar to that of *Sphenodon punctatus* (O'Donoghue, 1920)

and *Hemidactylus flaviviridis* (Mahendra, 1942) and differs markedly from that of *Varanus bengalensis* (Thapar, 1921; Bhattacharya, 1921) and *Uromastix hardwickii* (Bhatia, 1929). In *Varanus bengalensis*, the right spermatic vein opens into the vena renales revehentes of its side instead of into the postcaval. In *Uromastix hardwickii* "the right testis is supplied with four venae spermaticae, two of which open into the transverse anastomosis and the rest into the posterior vena cava".

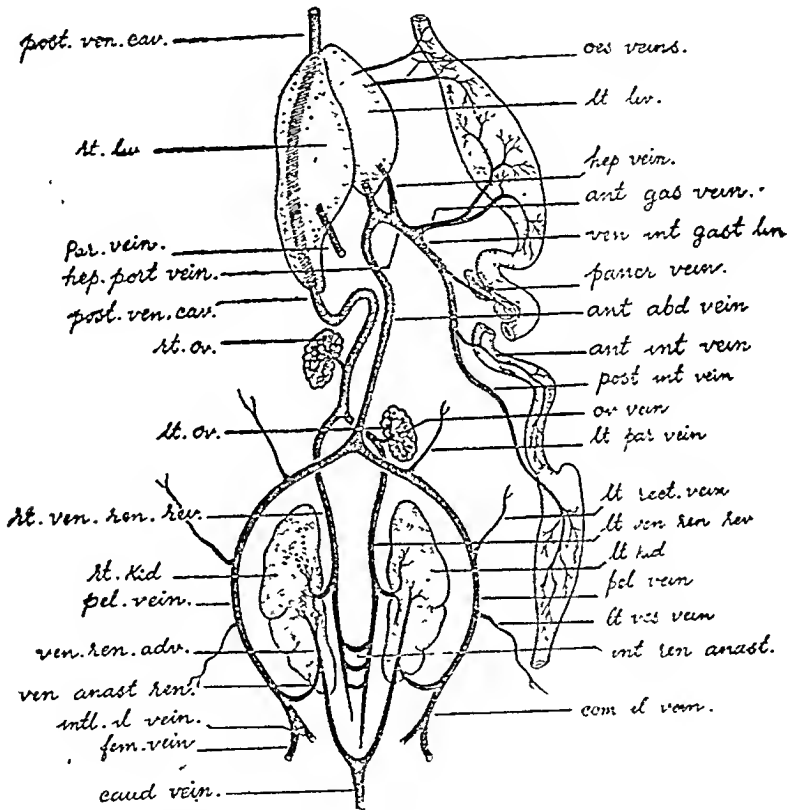


FIG. 5. *Riopa guentheri*—The posterior veins and the hepatic portal system.

ant. abd. vein, anterior abdominal vein; ant. gas. vein, anterior gastric vein, ant. int. vein, anterior intestinal vein; caud. vein., caudal vein, com. il. vein, common iliac vein, fem. vein, femoral vein; hep. port. vein, hepatic portal vein; hep. vein, hepatic vein, intl. il. vein, internal iliac vein; int. ren. anast., inter-renal anastomosis; lt. kid., left kidney; lt. liv., left liver lobe; lt. ov., left ovary; lt. par. vein., left parietal vein; lt. rect. vein, left rectal vein; lt. ven. ren. rev., left vena renales revehentes; lt. ves. vein., left vesicular vein; oes. veins, oesophageal veins, ov. vein, ovarian vein; pancr. vein, pancreatic vein; par. vein, parietal vein; pel. vein, pelvic vein; post. int. vein, posterior intestinal vein; post. ren. cav., posterior vena cava; rt. kid., right kidney; rt. liv., right liver lobe; rt. ov., right ovary; rt. ven. ren. rev., right vena renales revehentes; ven. anast. ren., vena anastomotica renales; ven. int. gast. lin., vena intestino-gastro-linealis; ven. ren. adv., vena renales advehens.



The two ovaries of *Riopa guentheri* are situated at different levels, the right one being anterior to the left. As in the case of the testes the left ovarian vein opens into the vena renales revehentes of its side while that of the right opens into the postcaval (Fig 5, *ov vein*)

As in *Hemidactylus flaviviridis* (Mahendra, 1942) the supra renal veins are minute and open into the venae renales revehentes of their respective side. In *Sphenodon punctatus* (O'Donoghue, 1920) and *Uromastix hardwickii* (Bhatia, 1929) these veins open into the gonadal veins

Blood from the tail region is collected by the caudal vein (Fig 5, *caud vein*) which runs the entire length of the tail, ventral to the caudal artery. At the level of the cloaca it bifurcates into two venae renales advehens (Fig 5, *ven ren adv*) or the renal portal veins which ramify within the substance of the kidney. This bifurcation of the caudal vein may occur prior to its entering into the conjoined end of the two kidneys as in *Sphenodon punctatus* (O'Donoghue, 1920) or after entering it as in *Hemidactylus flaviviridis* (Mahendra 1942) and *Riopa guentheri*

A common iliac vein (Fig 5, *com il vein*) is formed by the fusion of the femoral and internal iliac veins (Fig 5, *fem vein*, *int il vein*). The common iliac vein receives a small transverse vessel called vena anastomotica renales (Fig 5, *ven anast ren*) from the renal portal vein and then proceeds cephalad as the pelvic vein (Fig 5, *pel vein*). The pelvic vein is joined by three small veins the parietal vein bringing blood from the body wall, the rectal from the rectum and the vesicular from the urinary bladder (Fig 5, *lt par vein*, *lt rect vein*, *lt ves vein*)

Beyond the level of the kidneys, anteriorly, the two pelvic veins join together to form the anterior abdominal vein (Fig 5, *ant abd vein*). The anterior abdominal vein runs cephalad through the fat bodies receiving minute branches from them and finally joins the hepatic portal vein to enter into the left lobe of the liver

### THE HEPATIC PORTAL SYSTEM

Blood from the alimentary canal is collected by various veins which ultimately join together to form the hepatic portal vein (Fig 5, *hep port vein*). The following vessels go to form the hepatic portal system.

Two oesophageal veins (Fig 5, *oes veins*) emerge, one behind the other, near the posterior end of the oesophagus. Each oesophageal vein is formed by two branches coming from opposite directions on the wall of the oesophagus

Both the oesophageal veins open independently into the liver on its anterior dorsal aspect.

As in *Hemidactylus flaviviridis* (Mahendra, 1942) in *Riopa guentheri* also the hepatic portal vein is formed by the union of two large vessels, namely, the anterior gastric vein (Fig. 5, *ant. gas. vein*) and the intestino-gastrolinalis vein (Fig. 5, *ven. int. gast. lin.*).

The anterior gastric vein drains blood from the anterior part of the stomach and opens into the hepatic portal vein before the latter receives the anterior abdominal vein. In some specimens a small vein coming from the stomach is seen to join the anterior abdominal vein instead of the hepatic portal vein.

The intestino-gastrolinalis vein is formed by the union of several veins that bring blood from various parts of the alimentary canal posterior to the stomach. These veins can be called, according to the site of their origin, the pancreatic, duodenal, anterior and posterior intestinal and rectal veins (Fig. 5, *pancr. vein*; *ant. int. vein*; *post. int. vein*). Along with the above-mentioned veins there is a small vein bringing blood from the spleen and joining the hepatic portal vein. A hepatic vein (Fig. 5, *hep. vein*) coming from the liver joins the hepatic portal vein in front of the anterior gastric vein.

A parietal vein (Fig. 5, *par. vein*) bringing blood from the ventral aspect of the vertebral column joins the right liver lobe directly on its dorsal aspect.

#### THE PULMONARY VEIN

The common pulmonary vein (Fig. 2, *pul. vein*) is formed by the union of the right and left pulmonary veins as in *Hemidactylus flaviviridis* (Mahendra, 1942). Each pulmonary vein is formed by several small vessels that collect blood from the lung. It leaves the lung slightly anterior to its middle part, mesially. The common pulmonary vein runs parallel to the left pulmonary artery, crosses the left precaval vein and opens into the left auricle on its dorsal aspect.

#### CONCLUSIONS

The study of the vascular system of *Riopa guentheri* reveals that it is more or less primitive by virtue of the following:

The anterior position of the heart; tracheal veins ending in a capillary network on the trachea; large size of the ductus caroticus; absence of anastomosis between the two tracheal veins and the tracheal vein joining the

base of the precaval vein while the subclavian vein opens into the base of the large azygos vein instead of opening into the common jugular vein

There are not many adaptive features in the vascular system. Two such features that may be recorded here are (1) the reduction in size and importance of the subclavian artery and vein as a consequence of the reduction in size of the forelimbs and (2) the conspicuously large size of the azygos vein, probably, in harmony with the active habits of the lizard and also its fossorial mode of living both of which may demand better respiratory movements of the thoracic region

### SUMMARY

1 The heart of *Riopa guentheri* is situated slightly in front of the line joining the two axilla. It is moderately elongated and bears a gubernaculum cordis

2 The pulmonary artery gives off a tracheal artery as in some of the primitive lizards

3 The ductus caroticus is larger than the carotid trunk in size, a feature which may be looked upon as being primitive

4 The subclavian arteries arise at different levels and are very slender in correlation with the reduction in the size of the forelimbs

5 The various arteries supplying the alimentary canal arise at different levels along the course of the dorsal aorta. Of these arteries, the oesophageal and intestinal are double

6 There are about 25-35 pairs of segmentally arranged parietal arteries arising from the dorsal aorta and supplying the dorsal musculature and the body wall

7 The subclavian vein is a very thin vessel and opens into a conspicuously large azygos vein instead of opening into the precaval vein. This may also be correlated with the reduction in the size of the forelimbs

8 The right tracheal vein is larger than the left and there are no anastomoses between the two tracheal veins, a feature considered to be primitive

9 The venae renales revchentes are paired and arise between the kidney lobes. There are three anastomoses between them at their posterior end. Both are of equal calibre and contribute equally to the formation of the posterior vena cava

10. The caudal vein bifurcates into two venae renales advehens before entering the posterior ends of the kidneys.

11. The circulatory system of *Riopa guentheri* shows some primitive features like the anterior position of the heart, large ductus caroticus, tracheal vein joining the base of the precaval vein, absence of anastomosis between the tracheal veins and the subclavian vein opening into the base of the large azygos instead of into the common jugular vein.

12. The circulatory system of *Riopa guentheri* resembles those of *Hemidactylus flaviviridis* and *Sphenodon punctatus*.

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# THE ANATOMY AND HISTOLOGY OF THE ALIMENTARY CANAL OF AN OMNIVOROUS FISH *MYSTUS* (=*MACRONES*) *GULIO* (HAM.)\*

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## INTRODUCTION

STUDY of the alimentary canal of fishes is of special interest since it exhibits a higher degree of variation than that of any other group of vertebrates. The teleosts particularly show a good deal of diversity in their food and feeding habits and the correlation of food and feeding habits is of great interest because of this diversity.

Several studies have been made to correlate the structure of the alimentary tract with the feeding habit. A recent review of the literature has been given by Barrington (1957). The present work deals with the anatomy and histology of the alimentary canal of *Mystus gulio* (an omnivorous fish), *Tilapia mossambica* (a herbivorous fish) and *Megalops cyprinoides* (a carnivorous fish). This paper presents a concise account of the alimentary canal of *Mystus gulio* and will be followed by other papers.

## MATERIAL AND METHODS

The investigation was confined to freshwater (occasionally brackish) forms because of the greater ease with which they could be obtained. They were kept in the laboratory for about three months, when they were observed with regard to their feeding habits. Only freshly preserved material was used for histological purposes. Standard fixatives and staining techniques were used throughout the investigation.

### *Food, Feeding Habits and Gross Anatomy*

The stomach contents of about forty fish were examined and found to be mainly composed of crustaceans. A few algae and decaying leaves were

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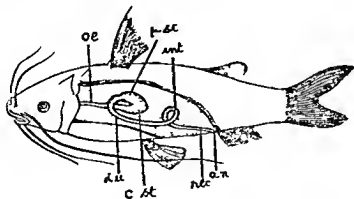
\* Part of the thesis approved by the University of Madras for the M.Sc. degree.

also noticed. In the laboratory they were fed on rice and earth worms. These facts go to show that the fish is omnivorous though animal food predominates.

A few specimens of the fish were kept in the laboratory under observation. These were not supplied with any food for about two months. During this period they were found to feed on algae that had made their appearance on small stones kept in the water.

The animal is a bottom feeder, when food materials are supplied, the fish does not try to get hold of them as they descend from the surface of the water, unless they are starved. The fish takes the food only after the food material has settled down at the bottom, except on rare occasions. According to Sarkar (1959) *Mystus seenghala* is a column feeder and piscivorous. But this species *Mystus gulio* should be considered as an omnivorous bottom feeder.

The several parts of the alimentary canal of the fish are the mouth, the buccal cavity, the pharynx, the oesophagus, the stomach, the intestine and the rectum (Text Fig. 1). The pyloric caeca are absent. The mouth is inferior. It is a crescentic slit. Each jaw has a crescentic patch of teeth medially within the lip margin. The teeth are villiform and homodont. There is also another crescentic patch, the vomerine patch, a little posterior to the maxillary crescent. The maxillary valve is between the two patches. There is also a U shaped mandibular valve.



TEXT FIG. 1

The gill arches are of variable length. The first arch has an anterior row of well developed gill rakers, the posterior row being absent. The same condition obtains in the second arch also except that the gill rakers are slightly shorter. The gill rakers of the other two arches are reduced to small knobs. The function of the gill rakers in this fish may be to protect the gill filaments from silt material and to some extent for filter feeding. Such

gill rakers have been described by Al-Hussaini (1946) in *Mulloides auri-flamma*.

Dorsally the pharynx has a pair of oval patches of teeth. Each one of these patches is carried on a rectangular whitish muscular region and can be moved for mincing the food. Between these oval patches there is a longitudinal depression. The median portion of the floor is raised into a ridge which fits into this depression. The posterior portion of this ridge includes the inferior pharyngeal bones of the last gill arches. The anterior parts of these inferior pharyngeal bones have narrow, elongated patches of teeth. The dorsal patches work against the ventral patches.

The function of the pharyngeal patches was observed in the laboratory. The mouth of the fish was kept open and with the help of a pair of pointed forceps, a small bit of a worm was placed near the oval patches. Immediately the patches began to move, obliterating the longitudinal depression between them and simultaneously the ventral pharyngeal patches were raised up and the food material was found to get pressed and swallowed.

Al-Hussaini (1949) mentions that the degree of development of the pharyngeal masticatory apparatus (horny pad, pharyngeal teeth) bears a direct relationship to the amount of plant food in the diet. But in this fish the buccal teeth probably prevent the prey from escaping and the pharyngeal patches crush it before it is swallowed. The fact that the prey is not masticated is shown by the stomach contents, as the prey is only injured but not masticated. The bulk of the food material in the stomach was found to have had no effects of mastication.

The oesophagus is a little dorsoventrally flattened tube overlapped ventrally by liver lobes. The pneumatic duct opens into the posterior region of the oesophagus. It leads on to the U-shaped stomach that is differentiated into a 'corpus' portion and a pyloric portion, the latter being found ventral to the oesophagus. There is a well-developed constriction separating the pyloric region from the duodenum.

The intestine is not very long. It measures only 6.5 cm. in a fish 10 cm. in length. The duodenum is its widest part. There is no intestino-rectal valve or external constriction to demarcate. The rectum opens out through the anus situated in front of the anal fin.

### *Histology*

The lip consists of mucosa and submucosa (Plate IX, Fig. 1). The mucosa is made of stratified epithelium and basement membrane. The epithelium



has many layers of cells which are of different shapes in different places. Taste buds are present, which are flask shaped. Each measures about  $45\mu$  in length and  $30\mu$  in thickness. The taste bud has its basal side concave and the other sides convex and encloses a few elongated cells. The presence of taste buds indicates that the fish is able to sense the nature of the food before swallowing. The well developed gustatory faculty can be correlated with the bottom feeding habit. According to Al Hussaini (1949), "the abundance of taste buds is rather to be correlated with the way in which the fish secures its food than with its nature".

The buccal cavity has the mucosa (consisting of mucous epithelium, the basement membrane and stratum compactum) the submucosa and muscularis (Plate IX, Figs 2 and 3). The mucous epithelium is stratified consisting of 6 to 10 layers of cells, of which only the peripheral two or three layers and basal two or three layers are continuous. The middle region has cells which have been described as conspicuous cells or club cells or giant cells. They are seen arranged in a row in section. They are fairly numerous, particularly on the sides. The club cells in *Macrones vittatus* are few and in *Sacco branchus fossilis* scattered (Vanajakshi, 1938). While in *Rita rita* they are arranged in rows (Islam, 1951). The club cells are generally polygonal or oval. Each cell has a large nucleus with conspicuous chromatin network. The cells occupy three fourths of the space between the surface of the epithelium and the basement membrane. The epithelium has a few mucous cells which are spherical or oval. Their nuclei are situated basally on the concave surface. There are numerous taste buds. They are rare in *Macrones vittatus* (Vanajakshi, 1938) and few in *Mystus seenghala* (Sarkar, 1959). They are found over the papillary out pushings of the submucosa into the epithelium. The stratum compactum is found just below the epithelium. In the middle region, it is thicker, being as broad as the stratified epithelium. The submucosa is made up of connective tissue fibres and is areolar. In the posterior region of the buccal cavity where musculature is noticed the submucosa is a thin layer. The roof of the posterior buccal cavity has musculature. The floor shows no musculature. Even in the roof the musculature is confined to two areas on each side of the midfloor of the cranium.

As already stated there are two oral valves. They are composed of dorsal and ventral mucosae with a submucosa between (Plate IX, Fig 4). They have a number of taste buds.

The pharynx can be distinguished into an anterior, a middle and a posterior region. Vanajakshi (1938) has divided the pharynx of *M vittatus* into anterior and posterior regions. Histologically the anterior region

resembles more the buccal region (Plate IX, Fig. 5). The roof of the anterior pharynx has two projections on the sides which are prominent and turned towards the interior. They are mostly made of stratified epithelium and have a number of taste-buds. Their significance is not clear (Plate IX, Fig. 6).

The middle pharynx has low folds (Plate IX, Fig. 7). It consists of more layers of cells. There are numerous mucous cells (Plate IX, Fig. 8). Club cells are few in number, except on the sides. There appears to be a negative relation between the number of club cells and that of mucous cells. In the middle region where mucous cells are numerous, the club cells are few and on the sides where the club cells are numerous, the mucous cells are few. Further, the positions of mucous cells in the regions where they are numerous correspond to those of club cells in other regions where they are numerous. It is also noticed that a few club cells are in the process of getting converted into mucous cells. Taste-buds are abundant. Their presence in this region must be concerned with the more active role of the region in feeding. The stratum compactum is found only on the sides. A layer of circular muscle fibres, a layer of longitudinal muscle fibres and a few oblique muscle fibres are also noticed. They are all striated.

The posterior pharynx has a striking resemblance to the anterior oesophagus and differs considerably from the anterior and middle regions of the pharynx. At the summit of each mucosal fold there are a few goblet cells in the stratified epithelium. The mucous secreting cells are larger and attain the maximum development in this region (Plate IX, Fig. 9). Such an abundance of mucous cells has been noticed by Dawes (1929) in *Pleuronectes* and by Al-Hussaini (1947) in *Atherina* and also by a number of other workers. The taste-buds are fewer in number. The submucosa is very broad and is not distinguishable from the lamina propria. The muscularis consists of an outer layer of circular muscle fibres and inner groups of longitudinal fibres found in the meshes of the submucosa (Plate IX, Fig. 10). A few oblique muscle fibres are also present. All the muscle fibres are striated.

The oesophagus lies partly outside and partly inside the body cavity. The former portion consists of four layers, mucosa, lamina propria, submucosa and muscularis, the hinder portion consisting of an additional serous layer. The longitudinal folds of the pharynx become strikingly deeper. There are 12 to 16 primary folds. Some of the folds are thrown into secondary folds, increasing the surface of the mucosa (Plate IX, Fig. 12).

The edges of the mucosal folds of the anterior oesophagus have stratified epithelium, the sides having one or two layers of columnar epithelial cells

which are mostly mucous producing. There are a few goblet cells occurring at the tips of the folds (Plate IX, Figs 11 and 13). A few taste buds are noticed in the anterior oesophagus but none in the posterior oesophagus (Plate IX, Fig 13). They are absent in *Mystus seenghala* (Sarkar, 1959). Currey has found them in the anterior region of the oesophagus, while many others have noted their complete absence. The mucosa of the posterior oesophagus has a single layer of columnar cells (Plate IX, Fig 13). The cells measure about  $53\mu$  by  $11\mu$ . Goblet cells are found at the crests of the mucosal folds. Currey (1939) found a top plate covering the columnar cells. It has not been seen here. Club cells are also noticed in this region (Plate IX, Fig 13). Nothing is mentioned about club cells in *Macrones vittatus*. There is a basement membrane. The lamina propria consists of loose connective tissue fibres. It is highly vascular. The submucosa lies next to the lamina propria. The muscularis consists of an outer circular and an inner longitudinal layer, both of them formed of striated muscle fibres. The longitudinal muscle fibres are present in the meshes of submucosa. The serosa is thick and the cells are not clearly seen.

The passing of the oesophagus into the stomach can be recognized distinctly by histological features. There is an oblique line of demarcation between the two made of connective tissue (Plate IX, Fig 14). The deep arborizing folds of the oesophagus pass into low ones, having columnar epithelium with fan shaped apical cells. Along with this change the gastric glands appear and soon become numerous. Striated fibres of circular muscles and longitudinal bundles are also noticed. Therefore, the term "oesogaster" can be used to refer to this transitional region.

The stomach has 8 to 10 gastric folds (Plate IX, Fig 15). The thickness of the gastric epithelium (mucous epithelium) varies in different regions of stomach, depending on the degree of development of the gastric glands. Usually in the case of U shaped stomach it is common to divide it into two, the 'corpus' and pyloric regions. Generally, the gastric glands are present in the former and absent in the latter.

The 'corpus' or body of stomach consists of mucosa (superficial epithelium and glandular epithelium), lamina propria, submucosa, muscularis and serosa. The superficial epithelium of the mucosa is made up of columnar cells and has numerous crypts. The cells are closely arranged. They are columnar and trumpet shaped being slender at the base and broader at the free surface, measuring about  $7\mu$  at the free end and about  $30\mu$  in length. Many of the cells are gracefully curved. The cells at the bottom of the crypts

are shorter and assume cylindrical form. The nucleus is situated at the basal third of the cell.

With Mallory's stain the reaction of the cells is striking. The peripheral part of the cells stains slightly blue, while the narrow basal region stains slightly red, the nucleus staining deep. Besides, the peripheral part of the cell exhibits a reticulate appearance and shows a faintly purple reaction with thionin, indicating the presence of mucus.

The glandular epithelium consists of numerous elongated tubular glands opening into the gastric crypts. The glands are compactly arranged with thin extensions of lamina propria between them. This serves to bind the glands together. The gastric glands which are packed in bundles are similar in details except in length. They are simple and tubular with one end blind and the other open, the latter opening into the crypt (Plate IX, Fig. 18).

Each gland consists of a number of cells arranged round a tubular lumen. In a cross-section the cells appear polygonal and have central nuclei which are spherical. There are granular bodies in all the cells. All the cells are alike, not differentiated into oxyntic and peptic as in mammals. This condition has been reported in all the fishes. Probably the granular cells found here are zymogen-producing ones, the granules being zymogen granules. No differentiation of neck-cells was observed.

The lamina propria is a connective tissue consisting of fibres in the form of a network. These fibres extend into the spaces between gastric glands. Blood vessels also enter along with this connective tissue. Thus the lamina propria serves a threefold purpose, binding the glands, supporting the entire glandular tissue and carrying the blood capillaries into the glandular tissue. The submucosa is built of an areolar connective tissue. The muscularis consists of an inner circular layer and an outer longitudinal layer. Both consist of unstriated muscle fibres. The serosa is much thicker than that of the oesophageal wall.

The pyloric stomach differs in certain respects. The gastric glands are absent and the muscularis is much thicker. The circular muscle layer is considerably thicker measuring about  $275\mu$  (Plate IX, Fig. 17). The region just anterior to the pyloric valve has very thick circular muscles which form the pyloric sphincter. The pyloric valve is present at the place where the pylorus ends and the duodenum begins (Plate IX, Fig. 16). The valve is in the form of a few flaps (Plate IX, Fig. 16) and out pushings into the lumen of the duodenum. The lamina propria is thick and forms the major part of the flap....The circular muscles take part in the formation of the...flap.

A transverse section passing through this region shows the presence of a narrow layer of circular muscles which are helpful in the closure of the pylorus

The intestine has the same structure throughout, varying in minor details like the number and shape of the cells in the epithelium and the thickness of the musculature. The intestine consists histologically, of mucosa, lamina propria, submucosa, muscularis and serosa (Plate IX, Fig 19). The mucosa is thrown into a number of folds, longitudinal and transverse, giving a complicated reticulate appearance. The mucosa is simple. Only two type of cells are found, the columnar and the goblet cells (Plate IX, Fig. 20). The columnar cells form the major part of the epithelium. They are long and slender, tapering towards the base. The nucleus is large. Its presence always causes a bulging of the cell outline. The swollen portions of the neighbouring cells interlock serving to strengthen the epithelium. There is a top plate. The goblet cells are found scattered. There are a number of leucocytes and a few granular cells in the epithelium.

The rectum can be recognized due to its thicker musculature (Plate IX Fig 21). There is no intestinal rectal valve. The folds of the rectum are not as tall as those of the intestine. The circular muscles are a characteristic feature of the rectum, being about thrice as thick as the longitudinal layer.

### DISCUSSION

The present investigation based on stomach contents shows that *Mystus gulio*, though it takes in more animal food, is omnivorous. According to Al Hussaini (1947) there are certain features which are well adapted to the modes of feeding and the kinds of diet. In *M. gulio* the mouth is inferior and not protrusible and it is mostly a bottom feeder. There are jaw-teeth and pharyngeal teeth patches, as the fish takes in a mixed diet. The gill rakers are well developed on the first two gill arches. Probably there is filter-feeding to some extent. The alimentary canal is short as the bulk of the food consists of animals.

According to Al Hussaini (1949), "the histology of the teleostean" intestine is one of the simplest among vertebrates. The present investigation confirms this view. The taste buds are common structures. They are present in the lips, buccal cavity, the pharynx and the anterior oesophagus. The pharynx has the largest number of taste buds. The abundance of taste-buds has been correlated with the way in which the fish secures its food rather than to its nature. Accordingly there is a well-developed gustatory faculty in this fish. It is expected to be so, as the fish has to select its food from the bottom. The presence of numerous mucous cells is a common feature,

they are saccular or spherical. As mentioned by Barrington (1957), the buccal cavity and pharynx with mucous cells and taste-buds are concerned in the seizure, control and probably selection of food. The stratified epithelium of the buccal cavity is supported by a stratum compactum. It is as thick as the stratified epithelium except anteriorly, where it is absent. The musculature consists of circular and longitudinal fibers, the latter being in bundles in the sub-epithelial connective tissue. They are not continuous and therefore not in a definite layer as circular.

The mucosal folds of the oesophagus are much more branched than in the pharynx. The anterior part shows stratified epithelium which is gradually replaced towards the posterior by columnar epithelium. Taste-buds occur in the anterior part. The muscularis consists of an outer circular and an inner longitudinal layer. However, the longitudinal muscles do not form a continuous thick layer throughout. Currey (1939) found longitudinal bundles outside the circular in the carp. Dawes (1929) also found the same arrangement. Hence there appears to be no fixed order of succession of these muscle layers in oesophagus.

Some fish are mentioned as stomachless (Barrington, 1957). This condition appears to be unrelated to the nature of the diet of fishes. Since a stomach is present in most fishes, the absence of the structure may be secondary. In *M. gulio* the stomach is U-shaped. The gastric glands are present only in the 'corpus' region. The gastric glands are not differentiated into two types, oxyntic and peptic. The pyloric part has thicker musculature and no gastric glands.

The intestine is short, as pointed out already. There are tall mucosal folds which have mostly columnar cells and some goblet cells. There is no agreement among the authors in respect of the definition of the rectum or its histological character. In *M. gulio* the rectum has thicker musculature and a large number of goblet cells.

#### SUMMARY

The anatomy and histology of the alimentary canal has been described. The mouth is inferior and the fish is mostly a bottom feeder. Jaw-teeth and pharyngeal teeth patches are well developed. Taste-buds are present in the lips, buccal cavity, pharynx and anterior oesophagus. The pharynx is divisible into three regions. A transitional region termed the 'oesogaster' is present. The stomach is U-shaped and the gastric glands are present only in the 'corpus' part. There is no differentiation of glandular cells into oxyntic and peptic cells. The pyloric stomach has thicker musculature. A

pyloric valve is present. The intestine is short; the mucosa has only two kinds of cells, columnar and goblet cells. The rectum has thicker musculature and a large number of goblet cells.

### ACKNOWLEDGEMENTS

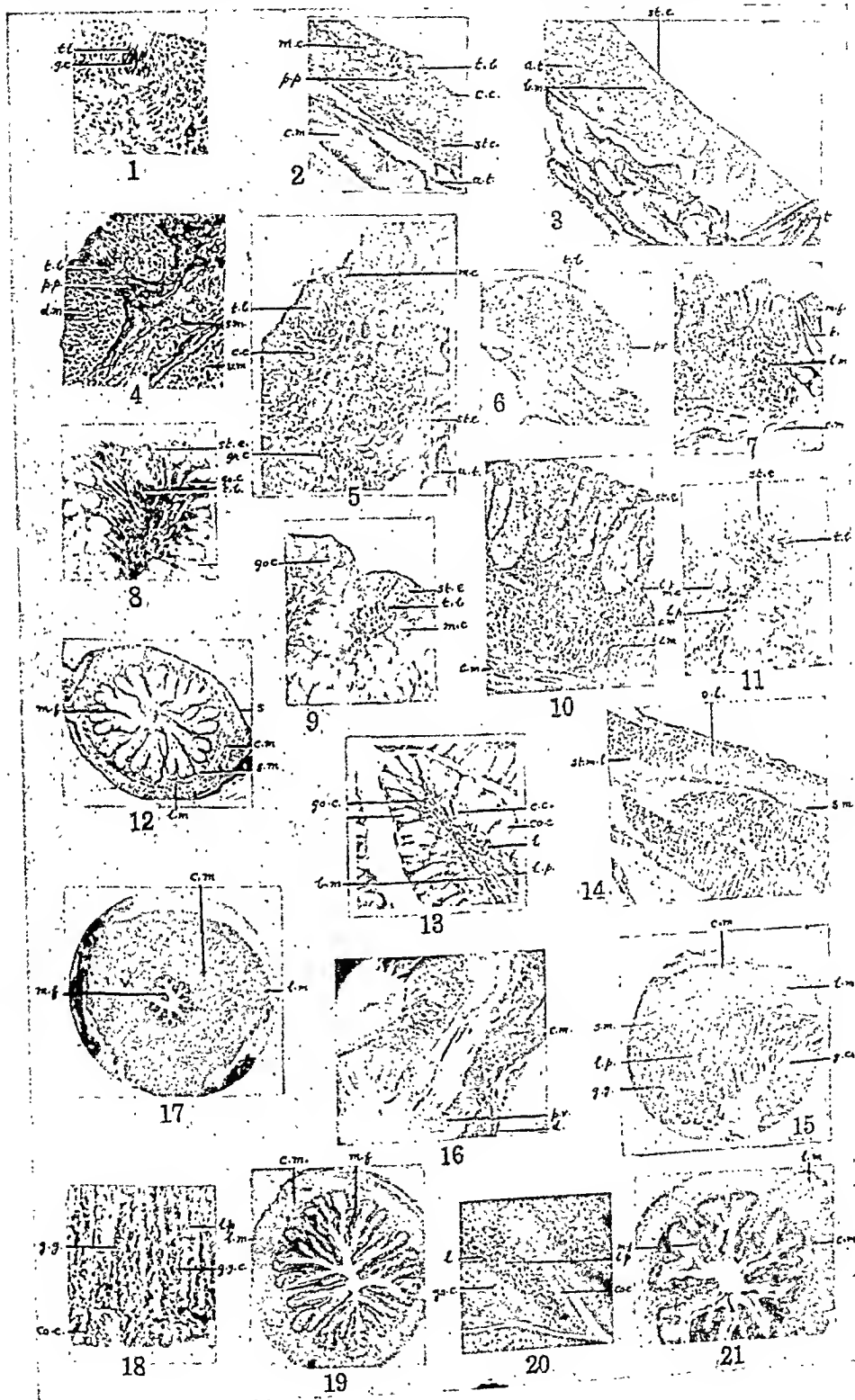
I am grateful to Prof. P. K. Menon, former Professor and Head of the Department of Zoology, Presidency College, Madras, for suggesting the problem and guidance.

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### EXPLANATION OF PLATE IX

- FIG. 1. T.S. of lip
- FIGS. 2. and 3. T.S. of Buccal membrane.
- FIG. 4. T.S. of maxillary oral valve
- FIG. 5. T.S. of Anterior pharynx.
- FIG. 6. L.S. of pharyngeal projection.
- FIG. 7. T.S. of Middle pharynx.



FIGS. 1-21



- FIG. 8. T.S. of Middle pharynx (part enlarged).  
 FIGS. 9 and 10. T.S. of Posterior pharynx.  
 FIG. 11. T.S. of Anterior oesophagus (part enlarged).  
 FIG. 12. T.S. of Oesophagus.  
 FIG. 13. T.S. of posterior oesophagus (part enlarged).  
 FIG. 14. L.S. of Oesogaster.  
 FIG. 15. T.S. of 'Corpus'.  
 FIG. 16. L.S. of Pylorus.  
 FIG. 17. T.S. of Pyloric stomach.  
 FIG. 18. T.S. of 'Corpus' (part enlarged).  
 FIG. 19. T.S. of Intestine.  
 FIG. 20. T.S. of Intestine (part enlarged).  
 FIG. 21. T.S. of Rectum.

#### ABBREVIATIONS

*a.n.*, anus; *a.t.*, areolar tissue; *b.m.*, basement membrane; *c.c.*, conspicuous cell; *c.st.*, 'corpus' (stomach); *du.*, duodenum; *g.c.*, gustatory cell; *g.g.*, gastric gland; *g.g.c.*, gastric gland cell; *g.cr.*, gastric crypt; *go.c.*, goblet cell; *gr.c.*, granular cell; *int.*, intestine; *l.*, leucocytes; *l.m.*, longitudinal muscle; *l.p.*, lamina propria; *m.c.*, mucous cell; *m.f.*, mucosal fold; *o.l.*, oblique line; *oe.*, oesophagus; *p.p.*, papillary projection; *pr.*, projection; *p.st.*, pyloric stomach; *p.v.*, pyloric valve; *rec.*, rectum; *s.m.*, submucosa; *st.c.*, stratum compactum; *st.e.*, stratified epithelium; *st.ml.*, striated muscle layer; *t.*, teeth; *t.b.*, taste-bud; *v.m.*, ventral mucosa.

# MINERALOGY OF THE CALC-GRANULITES FROM SRIKAKULAM DISTRICT

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## INTRODUCTION

THE calc-granulites are one of the interesting members of the Khondalite series and are found in association with the manganese ores of Srikakulam District. The typical occurrences are in Garividi, Koduru, Devada, Duvvam, Jadavari, Gadabavalasa and Ramabhadrapuram (see Fig. 1). This communication deals particularly with the mineralogy of these rocks.

## MODE OF OCCURRENCE

The calc-granulites represent the calcareous members of the Khondalite series and are conformable with the associated quartz garnet sillimanite-graphite gneisses, quartzites, or manganese ores. They occur at the bottom of the sequence in the form of disconnected beds striking NE-SW. The granulites are folded all along Koduru-Devada-Duvvam—a belt about 6 km long. The folds pitch  $4^\circ$  in NE direction.

Where there is intense folding and distortion as in Devada the calc-granulites are streaked with felsic material and resemble streaky gneisses. These streaks are not always uniform in size or shape, sometimes they are 3 to 10 cm long and are found in oval or round forms.

There is a general lithological variation in these rocks, namely calcareous in Koduru and magnesian in Devada, represented by abundance of calcite and diopside respectively.

The calc-granulites are banded, the thickness of each band varying from 5 to 20 cm. The calc-granulites near Devada consist of garnet, diopside and calcite bands which alternate with quartz and feldspar.

## MINERALOGICAL COMPOSITION AND TEXTURAL FEATURES

The calc-granulites consist of calcite, wollastonite, diopside, garnet (probably grossularite), quartz, scapolite, orthoclase, microcline and albite,

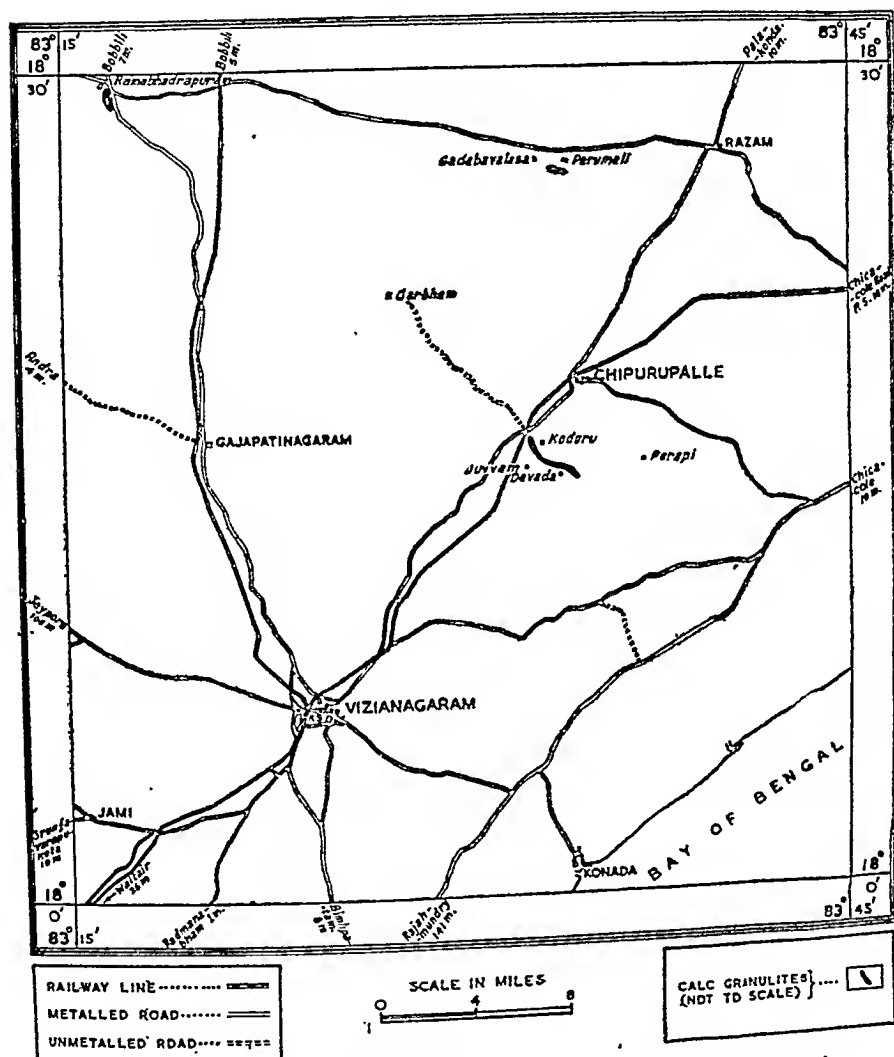


FIG. 1. Location of the calc-granulites in Koduru Area, Srikakulam District.

Apatite, sphene, muscovite, pyrite, serpentine, biotite and chlorite are found in minor amounts. The following grouping of the rocks is based on the important minerals and textures:

- (1) Quartz-rich varieties which are mainly gneisses to granulites. The quartz is porphyroblastic.
- (2) Calcite-rich varieties with gneissic texture, and if quartz is also in equal proportion to the calcite, the granulite texture is more predominant.

(3) The diopside rich varieties with bands each comprising either wollastonite or garnet or scapolite or calcite. These are medium grained and granulitic.

(4) The feldspar-rich varieties in which the granulite texture is predominant.

Table I gives the modal composition of the calc-granulites in the area.

TABLE I  
*Modal composition of calc-granulites*

Minerals	1	2	3	4	5
Garnet	4.79		10.90	.	..
Quartz		27.99	.	.	..
Orthoclase	10.00	40.71	.	2.90	25.82
Microcline			28.90		..
Diopside	13.58		4.89	.	17.30
Plagioclase	4.42		.		..
Scapolite	24.98	20.70	26.88	..	2.87
Wollastonite	18.20		..	.	5.90
Calcite	9.78	10.60	18.77	95.00	40.72
Sphene	2.70		5.26		4.17
Apatite	1.55		4.40	2.10	2.69

1 From hillock near Garividi Railway Station, Koduru.

2 From Koduru, Contact zone with intrusive (granite)

3 From Koduru mine. Contact with manganese ore.

4 Bore-hole sample from Koduru mine (60 m deep from ground level)

5 From Allikonda, south of Chintalavalasa.

The description of the constituent minerals is as follows

*Albite* is fresh in the sections and shows albite twinning. It is rarely intergrown with quartz. Small grains of diopside, sphene and apatite occur

as inclusions. Apatite occurs as needle-like inclusions which are arranged in parallel rows.

$2 V_z = 76^\circ$  to  $91^\circ$ : The anorthite content (Wahlstrom, 1955, p. 118) ranges from 2–20%; this indicates a low temperature of formation for such albite (Tröger, 1959).

*Apatite* in idiomorphic forms occurs in quartz: as small oval grains in wollastonite, scapolite, calcite and diopside. It is also found in the inter-granular spaces of quartz or in association with scapolite, diopside or sphene; innumerable needles of apatite are found also in orthoclase, albite and microcline.

Two generations of apatite could be distinguished; an idiomorphic early form of minute prisms and a later one of oval grains.

An interesting observation is the apatite-calcite association where the calcite has transformed to apatite along the borders.

*Calcite*, if abundant in the rock, is usually in the form of porphyroblasts; if diopside is abundant, the calcite is found segregated.

Calcite appears as filling material in cracks of the quartz. Rarely oval grains of apatite are seen as inclusions. Microscopic inclusions which could not be identified are distributed along the grain boundary of the calcite; sometimes plates of rutile are found parallel to the cleavages.

The calcite shows ferruginous and chloritic material along cleavages. Part of the calcite is scapolitised.

The *Potash feldspars* consist of orthoclase and microcline which are granoblastic.

Some of the potash feldspars contain acicular inclusions of apatite which are arranged in parallel rows. Microcline shows imperfect development of cross-hatched twinning.

Carlsbad twinning in orthoclase is rarely found.

$$2 V_x = 46^\circ \text{ to } 80^\circ$$

This variation in the optic axial angle indicates that the composition varies from  $Or_{50} Ab_{46.5} An_{3.5}$  to  $Or_{85} Ab_{14} An_1$  in their weight percentage (Wahlstrom, 1955, p. 102).

The *Quartz* is porphyroblastic in medium to coarse-grained rocks and in the finer, it is granoblastic.

Quartz shows *biaxial* positive character. The optic axial angle varies from  $4^\circ$  to  $17^\circ$ . This biaxial nature of quartz is not an uncommon feature according to Freund (1955, p. 122). This feature has been accounted as due to directional pressure phenomena at high temperature (Ramberg, 1958, p. 158; Schuller, 1948).

Scapolite occurs in the form of calcite sometimes preserving the cleavage and can be described as pseudomorph. Scapolite also occurs as segregations when it contains inclusions of calcite, apatite and diopside.

Scapolite shows high birefringence, like the quartz it is *biaxial*.

$$2V_x = 5^\circ \text{ to } 16^\circ$$

Sphene occurs as oval grains in association with diopside, wollastonite and scapolite. The sphene is rimmed by quartz.  $2V_z = 24^\circ$ , this suggests that the composition is pure calcium-titanium oxide without rare earths. Troger (p. 46) groups such a sphene as titanite with insignificant impurities of less than 1% of alumina and iron oxides. As has already been stated this is secondary from calcareous material and later than the granulite formation.

Wollastonite is elongated, sometimes platy and fibrous. Rarely twins are present (100 plane). The optic axial angle ( $2V_x$ ) varies from  $32^\circ$  to  $52^\circ$ , and the extinction on trace of *b*-axis varies from  $0^\circ$  to  $7^\circ$ .

The composition read from the optical characters is (Ca, Fe, Mn, Mg)  $\text{SiO}_3$ , according to Wahlstrom (p. 184). The variation suggests that it is bustamite.

#### PARAGENESIS OF CALC-GRANULITES

The calc granulites of Srikakulam District represent regionally metamorphosed impure calcareous sediments.

The mineralogical and textural studies indicate that diopside, calcite and quartz were formed in the granulite facies and that subsequently calcite was transformed into scapolite, sphene and apatite.

Since scapolite and quartz show similar anomalous biaxial behaviour it is suggested that subsequent to their formation they have been affected by directed pressure.

The granitic rocks which bear a discordant relationship to the calc-granulites of this area are found to be almost free from the effects of directed pressure. Also there are no major metamorphic effects by the granitic rocks on the calc-granulites.

### SUMMARY

The calc-granulites from Srikakulam District with particular reference to their field and mineral characters are described. Calcite, diopside, and quartz belong to the granulite facies while sphene, scapolite and apatite have been formed by the transformation of calcite during regional metamorphism.

### ACKNOWLEDGEMENTS

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# THE PECULIAR SCLEREIDS OF *CEPHALOTAXUS DRUPACEA* SIEB. ET ZUC. C.

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## INTRODUCTION

THE present paper is the fourth in a series of studies on the structure, distribution and ontogeny of sclereids in some conifers. Our observations on *Taxodium distichum* (Rao and Tewari, 1960), *Cryptomeria japonica* (Rao and Malaviya, 1963) and *Araucaria* spp (Rao and Malaviya, in press) have already appeared in press. The present paper deals with the peculiar, unbranched, variously shaped brachy and osteosclereids occurring in the stem and reproductive organs of *Cephalotaxus drupacea* Sieb et Zuc C. So far as we are aware, the occurrence of these sclereids much less their ontogeny has not yet been reported. Hardev Singh (1961), however, makes a passing reference to the sclereids in the seedcoats of *Cephalotaxus drupacea*, as just thick walled pitted cells in the hypodermal region. Seward (1919) who was one of the earliest to record the presence of thick-walled idioblasts in some conifers also did not make any specific reference to these sclereids in *Cephalotaxus*. It is likely that these sclereids have been missed by earlier workers as they are few in number and make their appearance after a fairly late stage in the development of the plant, after the secondary activity has started.

## MATERIAL AND METHODS

The material investigated was taken from the departmental collection. Foster's technique (1946) was used for clearing. The seeds were very hard so after removing the outer fleshy layer, they had to be kept in concentrated nitric acid for some time to be softened. Orange G in clove oil gave very good results while staining the younger parts for ontogeny, otherwise safranin-light green combination was used. Sections were cut at a thickness of 10-15  $\mu$ .

### *Distribution of sclereids*

The sclereids are totally absent in the leaves. They are, however, present though in small numbers in the stem. They appear, as has already been



stated, after secondary activity of the stem starts, and when the secondary phloem has differentiated. The sclereids occur mostly near the leaf traces in the cortex, and in the pith near the solitary central resin canal (Fig. 1 and Photo 1). They seem to have thus a rather localised distribution.

In the male cone, the sclereids occur in larger numbers as compared to the stem. They occur in the two sterile sporophylls at the basal region of the cone and also in the cone-axis (Fig. 2). They are absent in the fertile sporophylls.

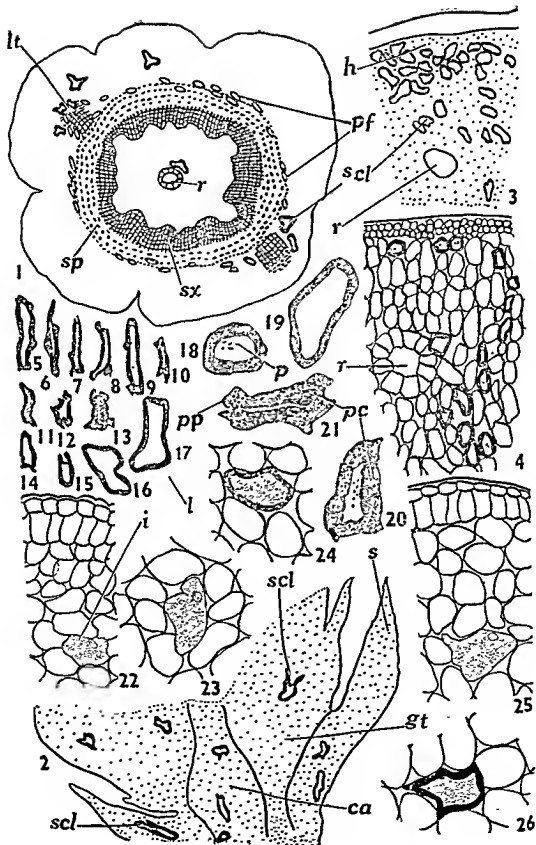
The female reproductive organ which is represented by one or two ovules in the axil of the leaf also shows greater number of sclereids in the outer fleshy layer of the seed (Figs. 3 and 4 and Photo 3). The sclereids occur throughout the thickness of the integument but are concentrated in the hypodermal layers. They become rather scarce in number towards the inner parenchymatous layer.

#### *Structure of the adult sclereids*

There can be distinguished mainly two types of sclereids in the genus *Cephalotaxus*. One type includes unbranched sclereids occurring more frequently in the cone-axis and sporophylls and less frequently in the stem (Figs. 5-10). These can be classed with the osteosclereids of Tschirch (1889) and Foster (1949). They have a narrow lumen and the ends of the sclereids are either very much elongated (Fig. 6) or are occasionally bifurcated (Figs. 7 and 10). The other type includes rounded or variously lobed sclereids with a broad or narrow lumen (Figs. 11-15). The sclereids found in the seedcoat belong to the second category. They are larger in size, have thinner walls, and a broad lumen (Figs. 16-17). They can be classed with the brachysclereids of Tschirch (1889) and Foster (1949) or "spheroidal sclereids" of Rao, T. A. (1957).

The secondary wall of the adult sclereid is very thick, lignified, lamellated, and is traversed by numerous unbranched (Fig. 18) or branched pit-canals (Figs. 19-20 and Photo 2). The pit apertures are either elongated and slit-like (Fig. 18) or are more or less rounded (Fig. 20). The adult sclereid is devoid of any protoplasmic contents in the lumen at maturity. The nucleus even though retained till a late stage of development, disappears ultimately in very old sclereids.

One curious feature of all the types of sclereids seen in *Cephalotaxus drupacea* is that in fully developed sclereids, small, pointed or blunt processes cover the wall (Figs. 8, 9 and 13). A careful study shows that these emer-



FIGS. 1-26

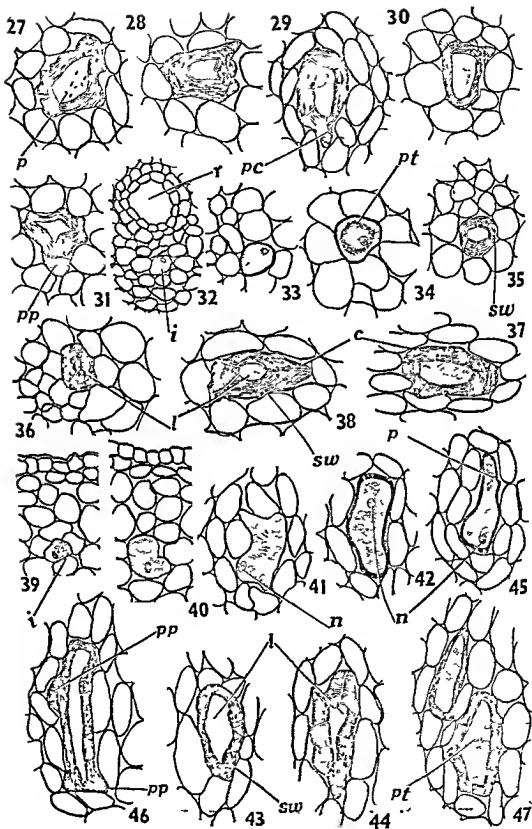
gences which generally number 3-5 for each sclereid are different from the lamellated wall and do not seem to be lignin as they do not answer to any lignin test. They appear to be the protoplasts of the original sclereid initial which have been cornered by the successive lignin layers (Fig. 21 and Photo 4). These localised original protoplast deposits, however, abut on the lamellated secondary wall and give an impression of being emergences. This is substantiated by these emergences like deposits taking a protoplast stain-like light green. They are not seen in all sclereids. These bulges also do not seem to be all in one plane.

#### *Ontogeny of sclereids in the stem*

The brachysclereids as well as osteosclereids of the stem, develop from a parenchymatous initial. This initial is seen in fully mature plants, when a few phloem fibres have already been cut off by the active cambium in the stem. A parenchyma cell in the cortical region forms the sclereid initial and this is recognisable by the presence of a distinct nucleus with a nucleolus, and very dense granular protoplasmic contents which are devoid of any vacuoles, and which occupy the entire lumen (Fig. 22). This initial does not undergo many changes. It just increases in size, without much change in the outline (Fig. 23) and very soon a secondary wall is laid down (Fig. 24). In other cases the increase in size is accompanied by slight protrusions of the primary wall (Fig. 25). Here also in the very next stage the protoplast starts the deposition of the secondary wall (Fig. 26). Gradually the lignin deposition increases, and the young sclereid becomes conspicuous against the surrounding parenchymatous background. The thickness of the secondary wall goes on increasing and the lumen is decreased (Figs. 27-29). In younger stages the wall does not show any lamellations but subsequently lignin is deposited in layers and the older sclereid shows a highly lamellated secondary wall. Pit-canals become prominent in the wall at about this stage (Fig. 29).

As stated already, in some of these sclereids while lignin is being deposited, some of the portions of the cell protoplast are cornered. They are pushed to a side and remain attached to the lignin deposits and are seen as emergences on the mature sclereid (Figs. 30 and 31). This has already been discussed above.

The sclereid initials in the pith may either differentiate near the solitary central resin canal (Fig. 32) or sometimes near the secondary xylem. The initial in the pith is of smaller size than that of the cortex. The nucleus does not have a very prominent nucleolus. The initial in other respects



FIGS. 27-47

resembles the initial in the cortex. There is an increase in size of the sclereid initial (Fig. 33) and the contents occupy the entire lumen. The secondary wall formation seems to be more clear in the sclereids of the pith region. Figure 34 seems to explain more clearly the development of the secondary wall. At this stage the protoplast assumes towards the periphery a dense granular appearance. It seems that this is the lignin, probably being secreted by the protoplast, and depositing itself on the outer limits of the protoplast and not on the primary wall which is quite removed from this. This is supported by lignin tests when the deposit takes up a dark red stain. Gradually, the primary wall disappears and the subsequent deposition of lignin builds up a thick secondary wall (Figs. 35 and 36). Our observations have shown that the secondary wall is not deposited directly on the primary wall but that it manifests itself from out of the protoplast at its periphery and later on thickens. This has been found in *Araucaria* sp. also (Rao and Malaviya, in press). Due to the thickness of this secondary wall the lumen is reduced to a very small structure (Figs. 36 and 38). Pit canals appear in the secondary wall. The sclereids in the region of the pith are very rarely lobed or ridged (Figs. 27 and 29). They more or less have a rounded or oval outline (Figs. 37 and 38).

The osteosclereids are more commonly found in the cone, although few brachysclereids may also develop here. The initial first appears in a sterile sporophyll slightly away from the epidermis (Fig. 39). There is a general increase in size (Figs. 40-41) accompanied by secondary wall formation (Fig. 42). Very soon the deposition of lignin starts and it increases gradually (Figs. 43-44). The nucleus has already disappeared by now. Sometimes one end of the sclereid becomes very narrow and meristematic (Fig. 45). The protoplasmic contents accumulate here and it invades through the surrounding tissue. Soon the sclereid becomes very long and the secondary wall becomes distinctly lamellated (Fig. 46). Occasionally in the cone region, the contents of the sclereid become discontinuous due to partial disorganization and gives the impression of small vacuoles in the lumen (Fig. 47), but ultimately however the entire contents disappear. The osteosclereids are not very highly elongated in the cones of *Cephalotaxus* as they are in the cones and leaves of *Araucaria* (Rao and Malaviya, in press). The brachysclereids are few here and their mode of development is exactly similar to those found in the stem.

In the seed the sclereids are found only in the seedcoat. They are brachysclereids and develop either from a hypodermal cell or from a parenchyma cell of the integument (Fig. 4). Their development is the same as that of the brachysclereids of the stem discussed above,

## DISCUSSION

An anatomical study of the fertile and sterile parts of *Cephalotaxus drupacea* has shown the occurrence in these parts, of peculiar, variously shaped unbranched brachysclereids and osteosclereids. A very peculiar feature of these sclereids is the presence of small, pointed or blunt projections over the secondary wall at certain places. These emergences judged by staining technique show that they are different from the lignin deposits of the secondary wall. They are here interpreted as part of the original cell protoplasts, which have been cornered during the lignification process. It is also possible that the process of lignification remains incomplete and permits the accumulation of the original protoplasts which have become dead and solidified into different shapes. So far as we are aware, this kind of emergences has not been reported before. While discussing the distribution of sclereids in *Araucaria* (Rao and Malaviya, in press) it was stated that the form of the sclereid, to some extent was partly influenced by the surrounding tissue. In the microsporophyll stalks where only elongated cells occur, the sclereid which after all is only a modified parenchyma cell can afford to elongate in the same plane in which the other cells of the stalk elongate. Thus elongated osteosclereids with blunt ends or with bifurcated ends occur abundantly in the microsporophyll stalks. We find that the same distribution and explanation for it also holds good in *Cephalotaxus*. As in *Araucaria* (Rao and Malaviya loc. cit.) so also in *Cephalotaxus* mostly lobed brachysclereids are found in the pith region, of the stem, where the parenchyma is compactly packed. On the other hand, in the cortex where the cortical cells are more loosely arranged with intercellular spaces, the sclereid initial can elongate into these and hence a few osteosclereids are produced. Although in the stem, both the brachy and osteosclereids are found, the brachysclereids dominate and in cones the osteosclereids are produced in larger numbers. It thus appears that the form of the sclereid is influenced by the surrounding tissue, and the nature of the organ in which they develop. As already stated, there are no sclereids at all in the leaf.

## SUMMARY

Peculiar, variously shaped osteo and brachysclereids occur in small numbers in the stem male cones and seed integument of *Cephalotaxus drupacea* Sieb. et Zucc. The distribution of these sclereids is rather localised in the stem and diffuse in the cones. A very curious feature of these sclereids is the presence of small, pointed or blunt emergences of the original cell protoplast, on the secondary wall. These are supposed to be

the unligified portions of the original protoplast, which have been cornered during the process of lignification. Both the types of sclereids develop from a parenchymatous initial. The ultimate form depends on the nature of the surrounding tissue and the organ in which they develop.

#### ACKNOWLEDGMENT

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\* Original not seen by us.

#### EXPLANATION OF FIGURE

Figs. 1-26. Fig. 1. A transverse section of an old stem showing the sclereid distribution,  $\times 28$ . Fig. 2. A longitudinal section of a male cone showing the basal region with sclereids,  $\times 28$ . Fig. 3. A longitudinal section of the seed,  $\times 28$ . Fig. 4. A part of above magnified to show the detailed structure,  $\times 40.4$ . Figs. 5-10. Various forms of osteosclereids,  $\times 64$ . Figs. 11-17. Various forms of brachysclereids,  $\times 64$ . Figs. 18-20. Macerated sclereids magnified to show the detailed structure,  $\times 144$ . Fig. 21. A macerated sclereid magnified to show the protoplasmic processes,  $\times 144$ . Figs. 22-26. The ontogenetic development of brachysclereid in the stem,  $\times 140$ .

*ca*, cone axis, *gt*, ground tissue, *h*, hypoderm, *i*, sclereid initial, *l*, lumen, *lt*, leaf trace, *p*, pits, *pc*, pit-canals, *pf*, phloem fibres, *pp*, protoplasmic process, *r*, resin-canal, *s*, sterile sporophyll, *scf* sclereid, *sp*, secondary phloem, *xx*, secondary xylem

Figs. 27-47 Figs 27-29 The later stages in the ontogeny of brachysclereid,  $\times 140$  Figs. 30-31 The protoplasmic processes are being formed,  $\times 140$  Figs 32-33 The ontogeny of sclereids in the pith region of the stem,  $\times 140$  Fig. 34 A stage showing the beginning of formation of the secondary wall Fig 35-38 Advanced stages in the ontogeny of sclereid in the pith,  $\times 140$  Figs 39-42. Development of an osteosclereid in the cone,  $\times 140$  Figs. 43-44. Stages after the lignification has set in,  $\times 140$  Fig 45 A developing sclereid showing one portion of it elongating,  $\times 140$  Fig. 46 The protoplasmic processes are formed in an osteosclereid  $\times 140$  Fig 47 In a later stage of development the protoplast giving an impression of vacuoles,  $\times 140$

*i*, sclereid initial, *l*, lumen, *n*, nucleus, *p*, pits, *pc*, pit-canals, *pp*, protoplasmic processes, *pl*, protoplast, *r*, resin-canal, *sw*, secondary wall

### EXPLANATION OF PLATE X

PHOTO 1 A portion of a transection of an old stem showing the sclereids in the pith region,  $\times 111.3$ .

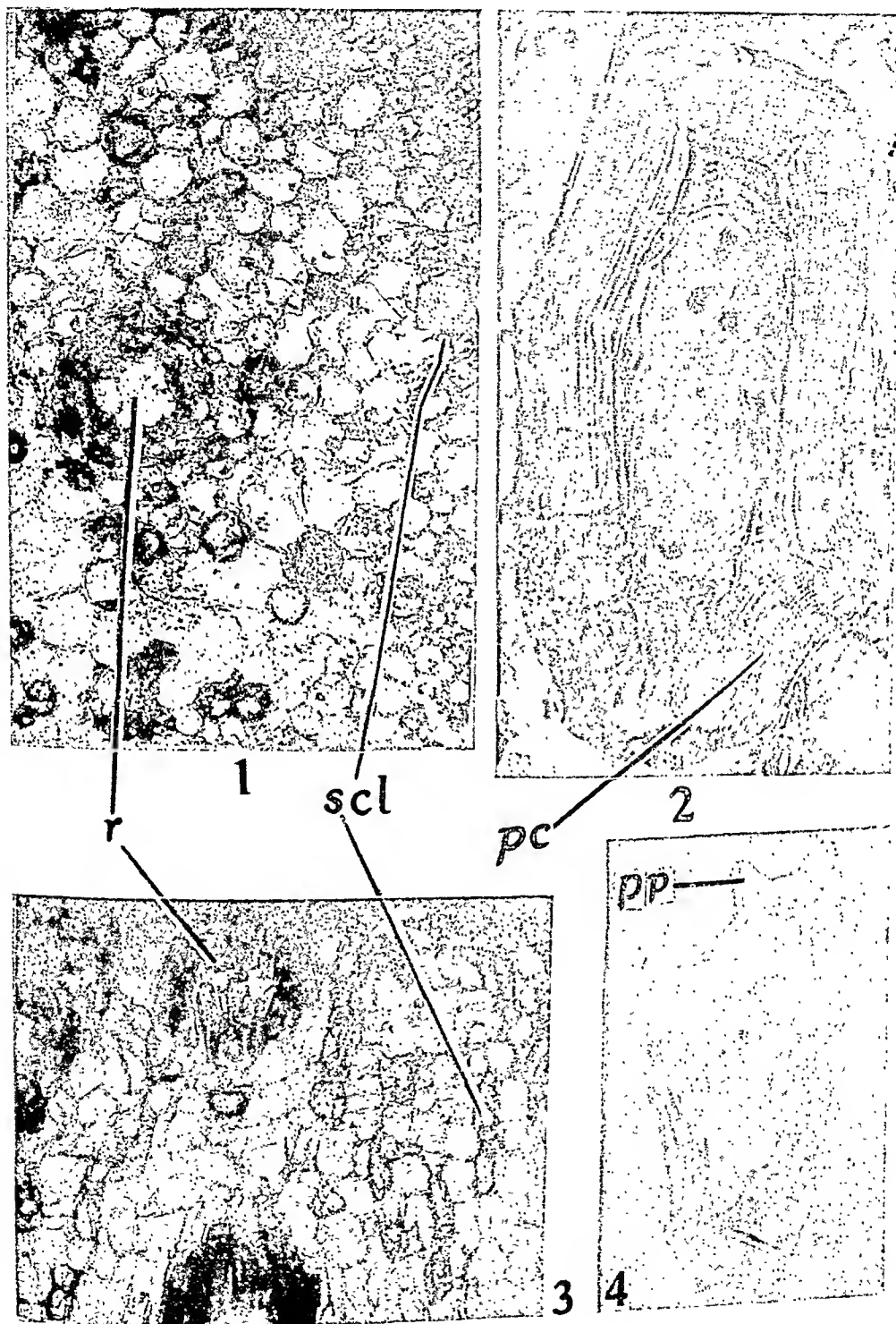
PHOTO 2 A macerated sclereid with lamellated wall traversed by pit-canals,  $\times 143$

PHOTO 3 A part of a longitudinal section of a seedcoat showing the sclereids,  $\times 615$

PHOTO 4 A macerated sclereid with protoplasmic processes,  $\times 498$

*pc*, pit-canals, *pp*, protoplasmic processes, *r*, resin-canal, *scf* sclereid





FIGS. 1-4

# MORPHOLOGICAL AND EMBRYOLOGICAL STUDIES IN NYMPHAEACEAE

## I. *Euryale ferox* Salisb.

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### INTRODUCTION

*Euryale* of the Nymphaeaceae combines interesting morphological and embryological features—primitive as well as advanced. Cook (1902, 1906, 1909) described the embryo-sac and embryo in a number of members of the Nymphaeaceae. Schnarf (1931) has reviewed the earlier embryological literature on the family, while Johansen (1950) has summarised the data on its embryogeny. Recently, Leinfellner (1956 *a, b*) and Moseley (1958) have described the structure of the stamens and its significance in the taxonomy and phylogeny of the family. However, no work has been done on the genus *Euryale* which bears cleistogamous flowers and so is interesting. *Euryale* was, therefore, undertaken for the present study.

### MATERIAL AND METHODS

The material was collected from Alwar, Rajasthan, India, in August and September, 1961-63. It was fixed in F.A.A and the usual methods of dehydration and embedding were followed. Sections cut 8-12 $\mu$  thick were stained with safranin-fast green and this gave excellent results.

### OBSERVATIONS

*Habitat.*—*Euryale* is a densely prickled aquatic annual with a perennating root-stock. The leaves are alternate, peltate (Figs. 1, 2) and when mature prickled on the under-surface.

*Flower.*—The floral bud is covered by soft spiny outgrowths (Fig. 3). The flower is cleistogamous and remains under water throughout. It bears four persistent spiny sepals (Fig. 4) and numerous small violet petals which remain closed throughout. The inner petals are lorate and the outer ones obovate (Fig. 7). The stamens are numerous (Fig. 6). Each of the outer

stamens is dorsiventrally flattened, having a deltoid base (Fig 5) and a distal appendage with embedded microsporangia. The inner stamens are of the conventional type. The change from the dorsiventrally flattened stamens to normal stamens is gradual and proceeds centrifugally.

The ovary is inferior, multicarpellary, syncarpous and 7-12 loculate (Figs 9, 10), with 2-3 ovules on parietal placentae in each locule (Fig 26). Usually, a single ovule matures in each chamber (Fig 27) and the others abort.

The fruit is a herry crowned with the persistent sepals (Fig 8). It is spinous and fusiform.

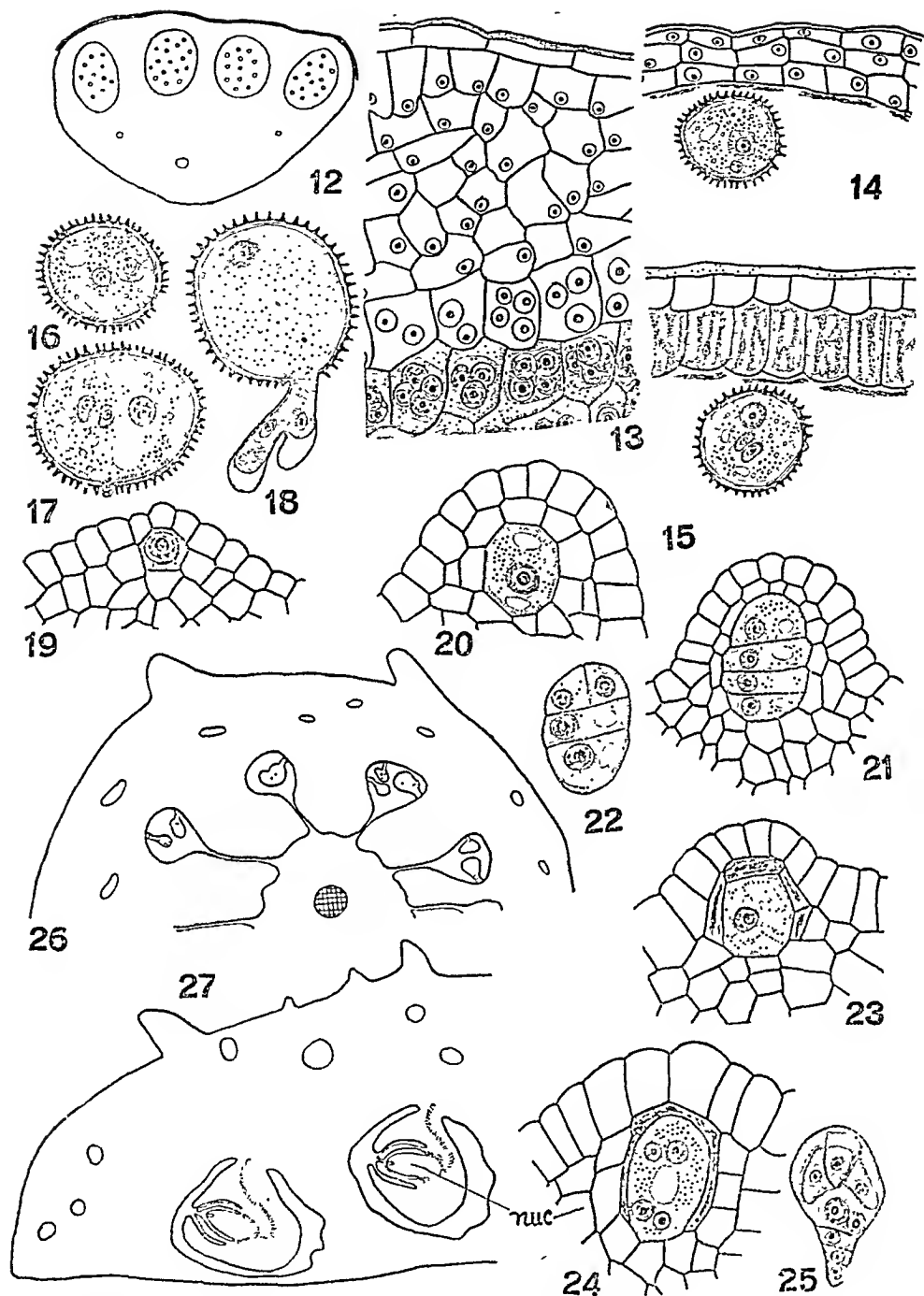
*Microsporangium, Microsporogenesis and Male Gametophyte*—As studied earlier, the outer stamens are dorsiventrally flattened (Fig 5). The microsporangia of these stamens are embedded in a massive sterile tissue. The anthers are four loculate (Fig 12) and become two-loculate at the time of dehiscence. Dehiscence is longitudinal and introrse.

The wall of the anther consists of an outer epidermis, an endothecium and 4-5 middle layers in the outer stamens (Fig 13). There are 2-3 middle layers in the inner stamens. The tapetum is uninucleate to begin with, but finally becomes multinucleate (Fig 13), it is of the secretory type and degenerates early (Fig 14). The endothelial cells elongate radially.

The microspore mother cells undergo meiosis resulting in the formation of tetrahedral (Fig 13) and isohilateral tetrads. The microspores are spherical and monocolpate. The exine is two layered, the outer layer being thick and tubercled (sexine, Erdtman, 1952) and the inner one thin and hyaline (nexine, Erdtman, 1952). The intine is also thin and membranous (Fig 16). The generative cell divides to form two sperm cells (Fig 17). The pollen grains germinate *in situ*. The pollen tubes of these grains are of variable length and carry the two sperm cells surrounded by the inner hyaline exine (Fig 18).

*Ovule*—The ovule is anatropous, bitegmic, crassinucellate and receives a single vascular strand which terminates at the chalaza (Figs 27, 31). The inner integument forms the micropyle. At the archesporial cell stage, four outgrowths arise from the funiculus which grow downward (Fig 31) and finally cover the seed, forming an aril. During their development, the outgrowths fuse along the greater length of their length and remain free only in the apical region where they consist of four valves (Fig. 11).

The nucellus is very massive (Fig 27). Its cells at the chalazal end and periphery are smaller in size as compared to those in the centre and the micropylar end at the eight nucleate stage of the embryo-sac.



FIGS. 12-27. Fig. 12. Cross-section of anther,  $\times 200$ . Figs. 13-15. A part of anther lobe,  $\times 1,620$ . Figs. 16-18. Pollen grains,  $\times 2,125$ . Fig. 19. Archesporial cell,  $\times 2,125$ . Fig. 20. Megaspore mother cell,  $\times 2,125$ . Figs. 21-22. Tetrads,  $\times 2,125$ . Fig. 23. Functional megaspore,  $\times 2,125$ . Fig. 24. Four-nucleate embryo-sac,  $\times 2,125$ . Fig. 25. Embryo-sac,  $\times 2,125$ . Figs. 26, 27. T.S. Ovary,  $\times 170$ .

*nuc*, nucellus.

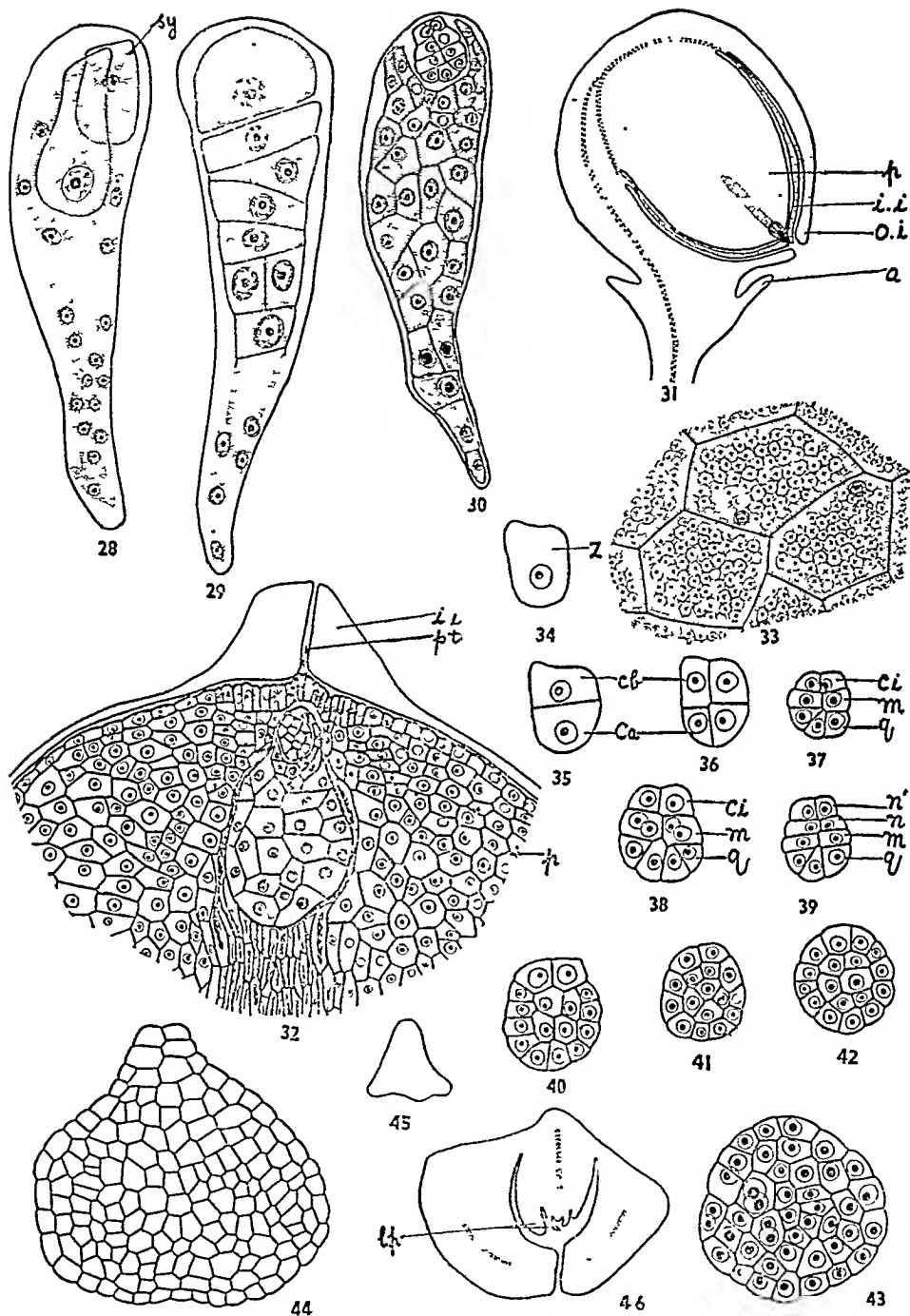
*Megasporogenesis and Female Gametophyte*—The hypodermal archesporial cell (Fig 19) divides to form an outer parietal cell and an inner megaspore mother cell. The parietal cell divides further antichinnally to form a single parietal layer (Fig 20). The mother cell undergoes meiosis and linear and T-shaped tetrads result (Figs 21, 22). The chalazal megaspore functions (Fig 23) to form the embryo sac. The nucleus of the functional megaspore undergoes three consecutive divisions to form an eight-nucleate embryo sac (Figs 24, 25). The organised embryo sac is minute in size as compared to the size of the nucellus. The synergids are large and conspicuous (Fig 25) and one of them persists after fertilization (Fig 28). The antipodal cells are small and usually degenerate before fertilization. Rarely, they are persistent. The embryo sac development conforms to the Polygonum type (Maheshwari, 1950).

*Fertilization and Endosperm*—Double fertilization is simultaneous. Remnants of the pollen tubes (Figs 31, 32) were observed at the micropylar end.

The primary endosperm nucleus undergoes free nuclear divisions (Fig 28). The chalazal end of the embryo sac elongates to form a tube like outgrowth (Figs 28–30) which penetrates deep into the nucellus bringing about the absorption of its tissue (Figs 31, 32). The wall formation is initiated at the micropylar end before the division of the zygote. Wall formation is progressive (Fig 29). The endosperm cells possess dense cytoplasmic contents in the beginning (Fig 30) but become highly vacuolated later on (Fig 32). The endosperm is almost completely consumed by the time the embryo matures.

*Perisperm*—The massive nucellus stores the food reserves and forms the perisperm in seed. At the globular stage of the embryo, the cells of the perisperm get enlarged towards the micropylar end in the centre. Those situated centrally may become binucleate also. In seeds with mature embryos these cells are full of starch grains. The number of starch grains varies considerably in the cells of the perisperm (Fig 33). They are comparatively a few in the cells of the micropylar end and those of the peripheral region, while the number is about a hundred in the centrally situated cells. The radially elongated cells of the micropylar region of the perisperm get cutinized on the outer and the radial walls. The perisperm forms the storage tissue in the mature seed.

*Embryogeny*—The zygote (Fig 34) divides by a transverse wall to form a terminal cell *ca* and a basal cell *cb* (Fig 35). *Ca* and *cb* divide vertically (Fig 36) and later on the derivatives of *cb* divide transversely forming *ci*



FIGS. 28-46. Fig. 28. Nuclear endosperm,  $\times 1,620$ . Figs. 29-30. Cellular endosperm. Fig. 29,  $\times 1,310$ . Fig. 30,  $\times 800$ . Fig. 31. Anatropous ovule,  $\times 90$ . Fig. 32. L.S. ovule showing inner integument, perisperm, endosperm and embryo. Mark the degenerating cells of perisperm,  $\times 1,000$ . Fig. 33. Enlarged cells of perisperm showing starch grains,  $\times 350$ . Figs. 34-44. Embryo development,  $\times 1,000$ . Figs. 45-46. Mature embryos,  $\times 800$ .

*a*, aril; *i.i*, inner integument; *i.p*, leaf primordia; *o.i*, outer integument; *p*, perisperm; *p.t*, pollen tube; *sy*, synergid; *z*, zygote,

and *m* (Fig 37) The derivatives of *ca* divide vertically forming a quadrant *q* (Figs 37-39) Subsequently, *cr* divides to form *n* and *n'* (Fig 39)

Further divisions in the embryo could not be followed closely but soon it becomes globular (Figs 40-43) The embryo gets flattened (Fig 44) and a cotyledonary ridge is formed which overhangs the young shoot apex Two saucer-shaped cotyledonary lobes (Figs 45-46) arise from the ridge Leaf buttresses are also organised forming leaf primordia in mature embryo (Figs 45, 46) In some ovules polyembryony was seen

Embryo development conforms to the Asterad type, Penea variation

### SUMMARY

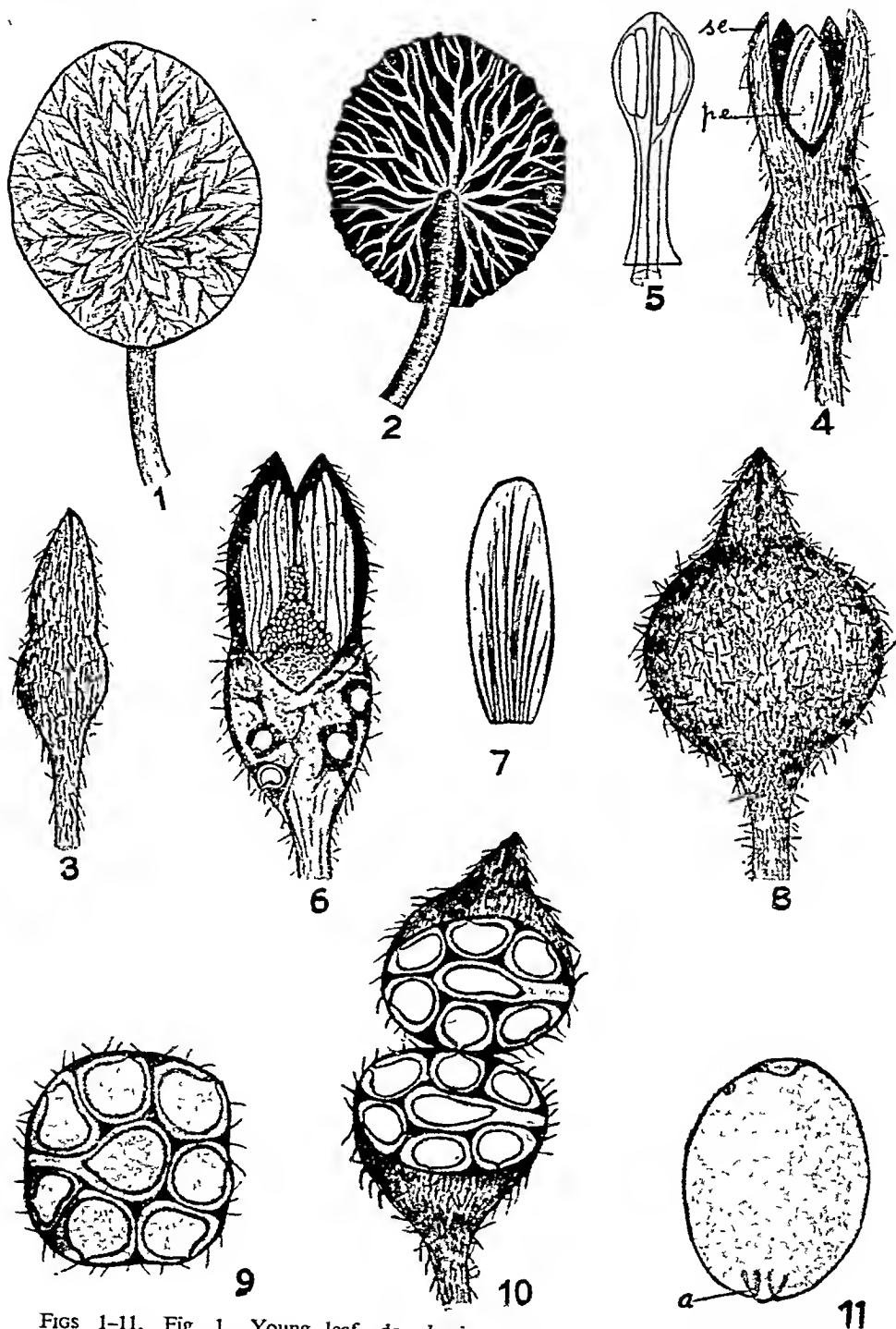
*Euryale*, a densely prickled aquatic annual, with a thick perennating root-stock, is survived by a single species only in parts of China, Japan, North America, and India

The leaves are alternate, peltate and prickled beneath Ep gynous flowers are submerged in water, having four spiny sepals, numerous small violet petals, numerous stamens—the outer dorsiventrally flattened with reduced and embedded microsporangia The ovary is multicarpellary, syncarpous, 7-12-loculate with parietal placentation

The anther wall consists of an epidermis, an endothecium, 2-5 middle layers and a multinucleate secretory tapetum The endothecium develops characteristic fibrous thickenings at the time of dehiscence The pollen grains are shed at the three-celled stage The outer exine is thick and tubercled and the inner exine is thin and hyaline The grains may germinate *in situ*

The ovule is anatropous, bitegmic and crassinucellate with a massive vascular strand Four outgrowths from the funiculus give rise to an aril The hypodermal archesporial cell cuts off an outer parietal cell and a megaspore mother cell Linear and T-shaped tetrads are observed The chalazal megaspore is functional, the embryo-sac is eight-nucleate and of Polygonum type

Double fertilization is simultaneous The endosperm is free nuclear Wall formation is progressive The endosperm is consumed by the time embryo matures The food material is stored in the perisperm in the form of starch The mature embryo possesses saucer-shaped cotyledons and 2-3 seminal leaves



FIGS 1-11. Fig. 1. Young leaf, dorsal view,  $\times 2$ . Fig. 2. Same, ventral view,  $\times 2$ . Fig. 3. Bud,  $\times 5.6$ . Fig. 4. Flower,  $\times 2.6$ . Fig. 5. Outer stamen,  $\times 90$ . Fig. 6. L.S. Flower,  $\times 3$ . Fig. 7. Obovate petal,  $\times 17$ . Fig. 8. Fruit,  $\times 2.5$ . Fig. 9. T.S. A part of fruit,  $\times 2.2$ . Fig. 10. T.S. Whole fruit,  $\times 2.2$ . Fig. 11. Seed showing aril,  $\times 19$ .

a, aril; se, sepal; pe, petal.



# ACKNOWLEDGEMENTS

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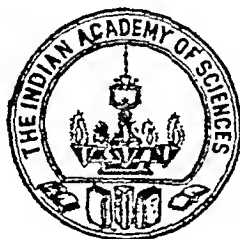
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# STANDARDIZATION OF $C^{14}$ STOCK SOLUTION AND FILTER EFFICIENCY IN THE COMPARISON OF PRIMARY PRODUCTIVITY MEASUREMENTS

BY R. RAGHU PRASAD, F.A.Sc.,\* P. V. RAMACHANDRAN NAIR  
AND J. J. A. McLAUGHLIN\*\*

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Received December 14, 1963

## INTRODUCTION

DURING the intercalibration trials on primary production conducted at the University of Hawaii, Honolulu, in September 1961, in which one of the authors (R. R. P.) also participated, factors influencing the variability of productivity measurements like sampling error, differential toxicity of samplers, sample treatment before incubation, inoculation, incubation, planchet preparation and counting were considered. Of these some of the factors such as sampling error and inoculation were made uniform by each group of scientists sub-sampling one and the same sample and all participants using the same  $C^{14}$  stock (C.S.I.R.O., Australia, No. 9). Different techniques were used only for incubation and two techniques were used for counting (for details see Doty, 1961).

The method for the measurement of primary production, followed in this Institute till recently, was on the lines marked out by the International Agency for  $C^{14}$  Determination at Charlottenlund, Denmark, including the computation of photosynthetic rates (see Steemann Nielsen, 1958). Since all the equipments used in this technique were not available at the time of the intercalibration tests at Hawaii, techniques used by participants from Australia, Japan, U.S.A. and U.S.S.R. only were compared. But later during July 1962 to June 1963 the first two authors conducted a series of parallel *in situ* experiments in inshore waters using the same C.S.I.R.O. stock of  $C^{14}$  ( $8 \mu\text{c}$ ), filters and filtering device as well as those of the International Agency ( $4 \mu\text{c}$ ). The counting of the planchets was done by the C.S.I.R.O. and the International Agency respectively.

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† Haskins Laboratories, New York, U.S.A., under U.S.P.H. GM-07022, support in part and U.S. Program, Indian Ocean Expedition.

The first two authors are thankful to Dr G F Humphrey and Dr H R Jitts of CSIRO, Australia, for the stock of  $C^{14}$ , filters and filtering apparatus and for also arranging the counting of the planchets.

## RESULTS

Twenty eight *in situ* experiments were conducted mostly in the inshore waters of Palk Bay using replicate samples containing natural population and under identical conditions. The results are given in Table I.

In the final values of primary production a 10% correction has been applied by the Agency for isotopic discrimination and respiration of  $C^{14}$ , whereas for values from CSIRO counting this correction has not been applied. Though any similarity in the activities of the two sets of planchets is not to be expected because of the difference in the strength of the stocks as well as the efficiencies of the counting systems, there should have been greater agreement in the ultimate values especially in view of the high sensitivity of the technique. The almost consistently lower rates in the CSIRO values cannot be due to the higher added activity since the strength of  $C^{14}$  is of no importance. Nevertheless, experiments were conducted with dilutions of 1, 2, 4, 5 and  $10\mu\text{c}$  from a Nuclear Chicago 0.5 millicurie stock solution of sodium carbonate and these gave proportional recovery of activity (173, 181, 410, 461 and 920 c.p.m. respectively) in a gas flow proportional counter. It may be mentioned here that the respective figures of 'added activity' at zero-thickness used in the computation of photosynthetic rates have been obtained by two different techniques (exponential extrapolation in the Agency technique and calculated from absolute activity in the CSIRO technique). According to Jitts and Scott (1961) the value obtained by exponential extrapolation can be low by 26% (subsequently corrected to 20% by Jitts, 1961) as compared to the latter method. The application of a lower value of added activity brings an overestimation in the production values. Hence it is obvious that the difference in approach in the standardization of stock is partly responsible for the observed disparity in the production values obtained by the two techniques.

Various methods have been used for the measurement of added activity, such as drying small aliquot of the stock solution on a planchet or converting to gaseous  $C^{14}O_2$  and determining in a gas counter and the more common method of determining indirectly from self absorption curves of  $BaCO_3$  planchets of varying thickness (*ref* Jitts, 1961). While discussing the merits and demerits of these methods Jitts (*op cit*) has remarked that though the extrapolation of self absorption curves can be made to be highly reproducible



TABLE I

Comparison of values of in situ experiments in Palk Bay obtained  
by the International Agency and C.S.I.R.O. techniques

Expt. No.	Date	Station		International Agency		C.S.I.R.O. Australia	
				c.p.m.*	mg. C/m. <sup>3</sup> / hour	c.p.m.†	mg. C/m. <sup>3</sup> / hour
1	26-6-1962	Off Mandapam	S	10,921	60.175	36,360	22.875
2	"	"	B	1,576	8.684	7,412	4.663
3	4-7-1962	"	S	32,122	132.760	24,915	11.756
4	"	"	M	7,476	30.898	43,568	20.557
5	"	"	B	720	2.975	3,663	1.728
6	9-7-1962	"	S	18,863	77.961	76,564	36.126
7	"	"	M	731	3.021	2,881	1.359
8	"	"	B	514	2.124	2,102	0.992
9	11-7-1962	Off Thangachimadam	S	10,620	43.892	27,897	13.163
10	"	"	M	2,019	8.344	10,294	4.857
11	"	"	B	122.6	0.507	907	0.428
12	"	"	S (D)	93.6	0.258	783	0.246
13	"	"	B (D)	71.6	0.197	726	0.228
14	18-7-1962	Off Athangarai	S	5,553	30.791	27,832	15.759
15	"	"	M	2,565	14.223	13,809	7.819
16	"	"	B	396	2.196	1,432	0.811
17	"	"	S (D)	122.6	0.338	434	0.137
18	"	"	B (D)	37.1	0.102	470	0.148
19	4-9-1962	Off Vizhingam	S	1,984	7.55	12,652	5.97
20	"	"	15 m.	299	1.14	1,358	0.64
21	"	"	30 m.	67.5	0.26	296	0.13
22	11-6-1963	Off Mandapam	S	1,291	7.31	10,692	5.05
23	"	"	M	1,043	5.90	10,018	4.73
24	"	"	B	50.2	0.28	483	0.23
25	17-6-1963	"	S	2,067	11.70	16,493	7.78
26	"	"	4 m.	3,235	18.31	23,738	11.20
27	"	"	8 m.	1,498	8.48	15,002	7.08
28	"	"	10 m.	626	3.54	6,863	3.24

\* Added activity at zero-thickness  $1.033 \times 10^3$  c.p.m.

† Added activity at zero-thickness  $8.67 \times 10^6$  c.p.m.

S, Surface; B, Bottom; M, Middlelayer; D, Dark bottle

TABLE II

*Comparative retention of activity on 3 types of filters of varying pore sizes*

Experiment No and Date	Type of filter and pore size		
	Membranfilter Gottungen (0.50 $\mu$ )	Millipore Type HA (0.45 $\mu$ )	Gelman Membrane Type AM 7 (0.30 $\mu$ )
1 7-8-1963			
Activity (c.p.m.)	2 445	2 574	2 964
mg C/m <sup>2</sup> /hour	3 8	4 0	4 6
Daily rate	45 6	48 0	55 2
% lost	17 3	13 0	.
2 8-8-1963			
Activity (c.p.m.)	8 303	8 434	(8 672)
mg C/m <sup>2</sup> /hour	12 92	13 13	(13 50)
Daily rate	155 04	157 56	(162 00)
% lost	4 3	2 8	
Recovered activity (c.p.m.)		238	
3 12-8-1963			
Activity (c.p.m.)	7 408	8 087	8 553
mg C/m <sup>2</sup> /hour	11 53	12 59	13 31
Daily rate	138 36	151 08	159 72
% lost	13 4	5 4	
Recovered activity (c.p.m.)	100	67	
4 17-8-1963			
Activity (c.p.m.)	51 649	56 900	47,326
mg C/m <sup>2</sup> /hour	83 74	92 25	76 73
Daily rate	1 004 88	1 107 00	920 76
% lost	9 2		16 8
Recovered activity (c.p.m.)		158	
5 17 8-1963			
Activity (c.p.m.)	12 882	13 839	15 985
mg C/m <sup>2</sup> /hour	20 89	22 44	25 92
Daily rate	250 68	269 28	311 04
% lost	19 4	13 4	
Recovered activity (c.p.m.)	70	86	

The figures in brackets in experiment 2 were obtained by filtering the filtrate from Millipore on Gelman filter after the original filtration on the latter went wrong.

and can be used for relative measurements of primary production it can introduce considerable errors when measurements using different counters are compared. The above series of experiments clearly illustrate this fact and stress the need for the adoption of a uniform method of standardization of the stock to make the results of various workers comparable. It is felt that the method suggested recently by Jitts and Scott (1961) which consists of determining the absolute activity of the C<sup>14</sup> added and the efficiency of the counter at zero-thickness and thereby the added activity could offset much of the difficulty and make comparison of values more feasible.

Another important aspect in productivity measurements, which has not received much attention, is the comparative efficiency of the filters commonly used by different workers in the retention of the activity. Lasker and Holmes (1957) studied the variability in retention of marine phytoplankton labelled with C<sup>14</sup> and concluded that it is advisable to employ filters with a porosity of at least  $0.45\mu$  and with relatively rapid flow characteristics. Recently Thomas (1961) has rightly drawn attention to the need of obtaining more knowledge of just how fine a filter is necessary to retain all or most of the radioactivity from C<sup>14</sup> labelled populations and also what negative pressure should be used for filtrations. During the intercalibration trials Millipore filters AA grade (pore size  $0.8\mu$ ) were used throughout. In the experiments mentioned above the authors used Millipore AA filters for the C.S.I.R.O. stock and Göttingen Membranfilter ( $0.5\mu$ ) for the Agency stock. AA filters retain less activity than HA filters ( $0.45\mu$ ) and considering the significance of the contribution of nanno-plankton in the primary production of these waters HA filters have been used for routine work in this Institute. Steemann Nielsen also has pointed out in a recent personal communication that for plankton algae like naked flagellates the filtration efficiency is an important factor.

In order to assess the relative efficiency of filters few experiments were conducted in August 1963 when the third author was here under the U.S. Program of Biology in connection with the International Indian Ocean Expedition. Natural populations were used and the retention of activity was measured on Millipore HA filters, Göttingen Membranfilter supplied along with the International Agency stock and also Gelman Membrane filters Type AM-7 having a smaller pore size ( $0.30\mu$ ).

Table II gives the results of these experiments.

It may be seen that excepting in experiment 4 when phytoplankton was very abundant in the sample, Gelman filters showed a higher retention of



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# ANATOMY OF THE PSEUDOCARP IN *ANACARDIUM OCCIDENTALE* L.\*

BY THOMAS M VARGHESE AND Y P S PUNDIR

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(Communicated by Prof V Puri, F.A.Sc.)

## INTRODUCTION

*Anacardium occidentale* L., a native of Brazil, is one of the economically important species of Anacardiaceae. The tree bears a pear shaped, yellow or reddish juicy 'fruit', the pseudocarp, which is the swollen pedicel and the disc. The true fruit is a reniform nut, borne on the distal end of the pseudocarp which together with the massive, plano convex cotyledons are edible. The former is of special significance to the plant as it aids in the dispersal of the hard nut by birds.

Some aspects of this interesting plant have been worked out. The works of Sieck (1895) on the development of resin ducts, Sarin (1938) on the physiological behaviour of the wounded leaves, Ventura and Hollanda (1959) on the amino-acids present in the cashew apple, Copeland (1961) on morphology, floral anatomy and a few aspects of embryology, are worth mentioning. But no detailed work on the developmental anatomy of the pseudocarp is available and this is the justification for the present attempt.

## MATERIAL AND METHODS

The pedicels of *Anacardium occidentale* at different stages of development were collected from Kerala State by one of us (T M V) and fixed in formalin-acetic-alcohol. Usual methods of dehydration and embedding were followed and sections were cut at 10-14  $\mu$  and stained with safranin fast green combination.

## OBSERVATIONS

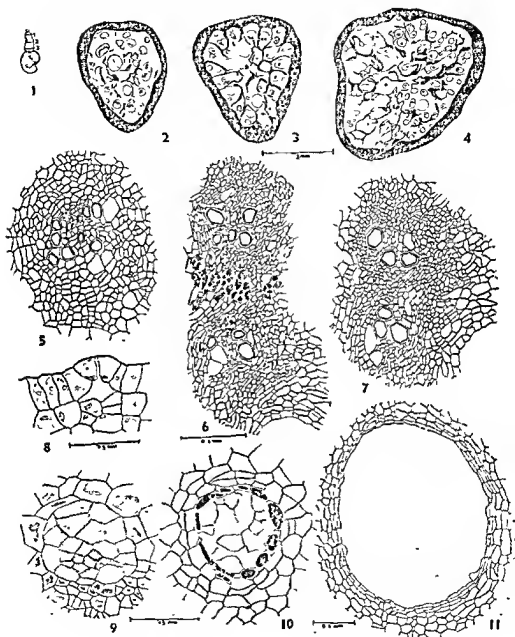
The young pedicel is slender and uniform (Fig. 1). But after fertilization it enlarges gradually together with the disc and form a succulent, juicy pseudo-

\* Research contribution No. 57 from School of Plant Morphology, Meerut College, Meerut, India.

carp commonly known as cashew apple measuring up to 42–45 mm. at the distal end (Figs. 12, 14, 17, 19 and 24).

Serial transections of young pedicels were examined. Below the unilayered epidermis which is interrupted by a number of stomata (Fig. 8), there are a few layers of cortical cells with tannin. At the basal portion of the pedicel the vascular tissue forms a dissected siphonostele which at a higher level divides into a number of bundles arranged in a ring. A few bundles appear in the centre also. The former as well as the latter divide and redivide to form a number of bundles at the upper portion (Figs. 2–4). The division of the bundles is quite interesting. The vascular elements in the bundle expand laterally and shift gradually in opposite directions in such a way that the protoxylem elements usually face each other. Later on these elements group themselves into two or rarely more units and get separated by tanniniferous cells to form two or more bundles with the sister bundles have their protoxylem elements usually facing each other (Figs. 5–7). Thus there is a gradual increase in the number of bundles from base upwards (Figs. 2–4). There are a number of resin ducts dispersed throughout the peduncle. Usually these ducts occur on the outer side of the vascular bundles (Fig. 4).

As Copeland (1961) has pointed out the occurrence of resin ducts is a characteristic feature of the family Anacardiaceae. It is, therefore, interesting to note the development and nature of these structures. The number of ducts is more at the terminal portion of the pedicel than at the base. In the developing pseudocarp the number of ducts increases. As has been noted by Harada (1939) in young fruits of *Rhus* and Venning (1948) in *Mangifera* small groups of special cells were distinguishable from the neighbouring cells by their thick contents, and have been designated as precursors of resin ducts (Fig. 9). These form long columns in the pedicel, usually on outer side of the phloem. The layer of cells just outside these group of cells attains dark contents (Fig. 10), while the layer encircling the cells with thick contents divides by tangential walls to form 3–4 layers of cells. The cell-walls of the 'precursor' group of cells disappear leaving the contents inside the canals. When the pedicel grows to maturity the cells of the endothelial layer with thick contents breakdown as they fail to keep pace with the enlarging duct. Three to four layers of radially elongated cells just outside this layer form the endothelium during the later stages, and their tangential walls become stretched as the growth of the pseudocarp is in progress (Fig. 11). The enlargement of resin ducts has a significant role in increasing the bulk of the pseudocarp. Copeland (1961) noted that the pith region of the pedicel does



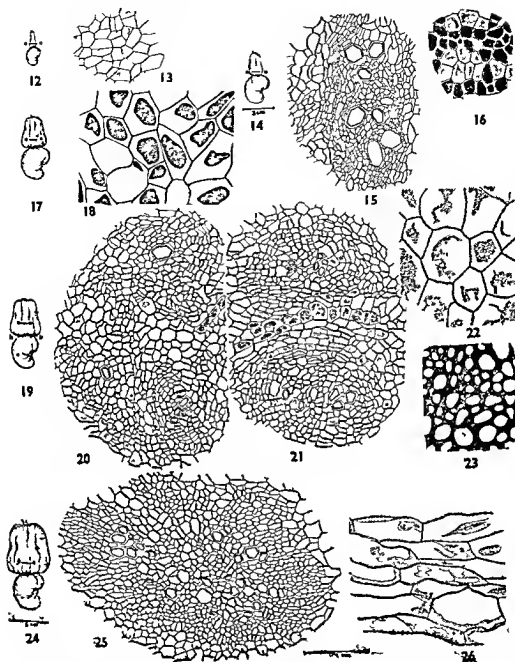
FIGS. 1-11. Fig. 1. The developing fruit with the pedicel. Figs. 2-4. The transection passing through the portions marked in Fig. 1 as 1, 2, 3 respectively, showing the increase in bundles, resin ducts and definite distribution of tannin. Figs. 5-7. The mode of division of bundle sheath cells. Fig. 8. Stoma in the epidermis of the pedicel. Figs. 9-11. The developmental stages of the resin ducts.



not contain any resin duct. But during the present study resin ducts were noticed in the pith region also.

The growth of the pedicel is an interesting point to be noted. The bundles in most portion of the pedicel are concentric with the phloem surrounding the xylem and protoxylem group facing each other (Fig. 15). It is to be noted that most of the cells in the bundles, except the xylem vessels have compressed walls when the growth starts. Some of the cells outside the xylem vessels are well marked with prominent nuclei which are the procambial cells. These cells form more or less a discontinuous ring (Fig. 15). As the growth starts, the walls of the cells are stretched out and the cells with prominent nuclei enlarge and divide repeatedly increasing the bulk of the bundle, towards the xylem as well as towards the phloem. In the growing pseudocarp some of the bundles at the distal end get divided into two or more segments and each one behaves as a separate unit and grows independently to form concentric bundles, thus contributing to the enlargement of the pseudocarp (Figs. 20-21). After the pseudocarp attains about 22-25 mm. in diameter (distal end) the bundles stop its growth and the parenchymatous cells of the ground tissue around the bundles enlarge considerably (Figs. 13, 16, 18, 22 and 26). The enlargement of all the cell-walls is equal in earlier stages but subsequently the radial walls grow longer. The resin ducts also enlarge considerably by the elongation of the radial walls of the endothelium and surrounding cells. A group of parenchymatous cells constituting the disc at the distal end of the pseudocarp where the nut is attached become lignified (Fig. 23). The lignification of these cells is significant as it fortifies connection of the nut with the pseudocarp and hence helping the dispersal of the nut to distant regions by birds.

In a growing pedicel considerable changes take place in the amount and distribution of tannin also. The tannin contents are less in the young pedicel (Fig. 13). They increase gradually in quantity and when the pedicel starts enlargement 8-10 layers of the cells in the cortex, just below the epidermis and most of the cells separating the bundles are completely filled with tannin. Up to this stage the distribution of tannin follows a definite pattern (Fig. 16). Later on the tannin occurs in all cells except the vascular elements and the cells surrounding resin ducts (Fig. 18). After the pedicel attains 22-25 mm. in diameter the amount of tannin starts decreasing and when the pseudocarp approaches maturity, located only in the centre of the cells (Fig. 22). In mature pedicel (cashew apple) the tannin contents disappear completely and the cells become enriched with globular bodies probably glycogen (Fig. 26). At this stage the pseudocarp is edible.



FIGS. 12-26 Figs 12, 14, 17, 19 and 24 Developmental stages of the pseudocarp  
 Figs. 13, 16, 18, 22 and 26 Cells of the ground tissue from the portions marked 'a-a' in Figs. 12,  
 14, 17, 19 and 24 respectively Figs. 15 and 25 Bundles from the portion marked 'a-a' in Figs. 14  
 and 24 respectively showing the contents. Figs. 20 and 21. The bundles from the portion  
 marked 'a-a' in Fig. 19, for showing the segmentation and independent growth of the bundles.  
 Fig. 23. Lignified cells of the disc from the portion marked 'b-b' in Fig. 19 Note. The marked  
 cells in Fig. 15 are procumbent in natura.

## DISCUSSION

The developmental anatomy of the pseudocarp of *Anacardium occidentale* is interesting in several ways, especially the increase in the size of the pedicel, in the development of resin duct and changes in the tannin contents at different stages of development.

Increase in the size of the pseudocarp is due to three factors. The growth up to 20–25 mm. diameter is chiefly due to the enlargement of the bundles brought about by some of the procambial cells around the xylem elements. However, the bundles at the distal end of the pseudocarp breaks up into several groups and each of them grows independently, contributing to the size of the pseudocarp. When the growth of these bundles almost stops, the parenchymatous cells throughout the pedicel enlarge rapidly. The increase in number and size of the resin duct is another factor which contributes to the enlargement of the pseudocarp.

There has been some difference of opinion as to the mode of formation of resin canals in Anacardiaceae. Engler (1896) described them as schizogenous in the family Anacardiaceae, whereas Sieck (1895) who studied them in the mature fruits of *A. occidentale* referred to them as Schizo-lyigenous. Recent investigation of Venning (1948) showed that the development of these ducts is Schizogenous in the stems and leaves of *Schinus* and in the developing fruits of *Mangifera*. In the leaves and stems of *Spondias* and *Mangifera*, however, they develop Schizo-lyigenously. The development of resin ducts is lyigenous in the floral pedicels and receptacles in *Spondias* and *Mangifera*. The formation of resin ducts in the pedicel of *Anacardium occidentale* is lyigenous and thus resemble the condition in *Spondias* and *Mangifera*.

## SUMMARY

The present work deals with the changes during the development of the Pseudocarp in *Anacardium occidentale*, which is morphologically the pedicel and the disc.

The number of bundles increases from the base towards the distal end in a peculiar way, which has been described in detail. Some of the bundles appear to arise *de novo* in the pith whereas the others form a continuous system from base to top.

The development of resin ducts in the pseudocarp is described in detail. They develop lyigenously.

The growth of the pseudocarp is also dealt with. There are three factors which helps in the enlargement of the pseudocarp (1) segmentation

and enlargement of the vascular bundles, (2) the enlargement of the parenchymatous cells in the cortex and pith and (3) the increase in number of the resin ducts and their enlargement

The amount of tannin contents in the cortical and medullary cells was also studied. The tannin contents increase gradually up to the time when the pseudocarp is 22-25 mm in diameter. Thereafter, they reduce gradually and disappear, completely.

#### ACKNOWLEDGEMENTS

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# EFFECT OF IMPLANTATION OF ANDROGENIC GLAND ON THE RELATIVE GROWTH OF ABDOMEN IN *OCYPODA PLATYTARSIS* (M. EDWARDS)

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## INTRODUCTION

CHARNIAUX COTTON (1957) demonstrated that the implantation of the androgenic gland from the male into the female of *Orchestia gammarellus* brings about the transformation of not only the ovary into the testis but also of the female secondary sex characters into those of the male. The slender claw of the female of *Orchestia* is transformed into the powerful claw characteristic of the male. It is now known that the androgenic gland can bring about similar transformation in other Malacostracan forms.

The transformation of the secondary characters implies a change in relative growth coefficients. It is well known that the external sex differences are related to differences in growth rates in the two sexes. As Teissier (1960) pointed out, the techniques for study of relative growth enable us to state exactly the periods when the action of internal factors condition secondary sex differentiation. Weymouth, Donald and Mackay (1936) have shown that the relative growth coefficient in the male abdomen is greater than that of the female specimens. A similar condition has been studied in *Callinectes sapidus* by Newcombe *et al.* (1949). Prasad and Tampi (1954) calculated the relative growth coefficients in the abdomen of *Neptunus pelagicus*. These studies relate to the growth differences in normal sexes and not in the process of sex transformation.

Charniaux Cotton (1957 *a*) observed that females of *Orchestia* into which androgenic gland was implanted progressively acquired the male secondary characters during the post-operative intermoult periods.

The present account is a quantitative study of sex transformation in *Ocyroda platytarsis*. The effect of implantation of androgenic gland into the female *Ocyrod* has already been reported by the author (Sarojini, 1962).

In the present paper, the changes in relative growth coefficients during sex transformation resulting from implantation of the androgenic gland have been calculated, and these coefficients are compared with those of normal and female specimens

### MATERIAL AND METHODS

The specimens were collected locally near the mouth of the Vellar estuary at Porto Novo. To determine the relative growth coefficients in normal male and female, 40 male specimens and 35 female specimens were studied. For determination of relative growth coefficient during sex transformation, fifteen immature female specimens were implanted with androgenic gland and they were measured at intervals during the post-operative intermoult periods. The method of implantation, and the maintenance of the specimens have been described elsewhere (Sarojini, 1962). The width of carapace was taken as standard and represented on the abscissa in all cases. All measurements were taken with fine dividers and expressed in millimeters. The equation  $Y = bx^a$  employed here was first described by Huxley (1932). The formula

$$a = \left[ \frac{\log 1 + v_y^2}{\log 1 + v_x^2} \right]^{-1}$$

given by Kermack and Haldane (1950) was also employed, and a close agreement was found between the results obtained by the two methods.

### RESULTS AND DISCUSSION

The results of the present analyses of growth are given in Table I.

The results of the analyses are given in Table I. The relative growth coefficient for normal male is 1.588, for the female specimens it is 1.177.

It is observed that the growth rates also differ between the two sexes. The external sex differentiating characters are due to differential growth rates between the two sexes. The relative growth coefficient for the implanted specimens during the first post-operative intermoult period is 1.3, and during the second post-operative intermoult period it is 1.6 (Fig. 1). It will be seen that the growth coefficient increases by degrees. The difference in relative growth between normal males and the males resulting by transformation of female specimens is not significant.

The observations of Charniaux-Cotton (1957) on *Orchestia* relate to the slender claw of the female which, as the effect of implantation of the androgenic gland becomes transformed progressively into a powerful male

TABLE I

Variations in the relative growth coefficient of normal male, female and transformed female specimens of *Ocypoda platytarsis*

Sex	Number	Size	Relative growth coefficient
Normal female .. ..	35	> 40 mm.	1.177
Normal male .. ..	40	> 40 mm.	1.588
Female transformed into male specimens .. ..	15	> 35 mm.	1.6
		I phase	1.3
		II phase	1.6

't' for difference between normal male and female 0.9167;  $p$  0.200.

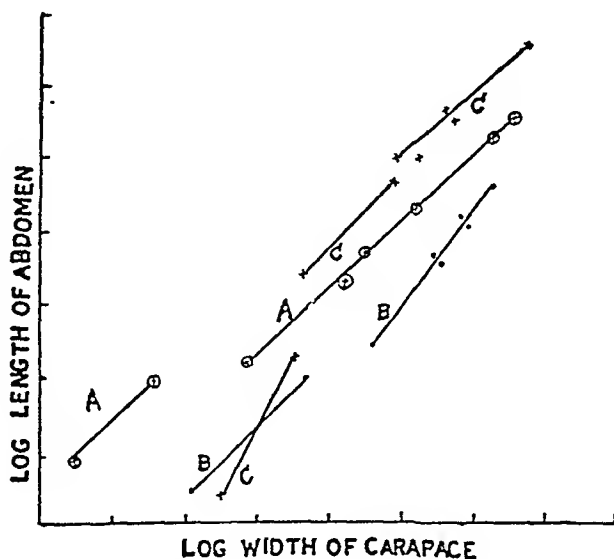


FIG. 1. Graph to show relative growth of abdomen in: (A) Male normal; (B) Female normal; (C) Female with androgenic gland implanted into it.

claw during the post-operative intermoult periods. The transformed claw finally assumes the form of the claw of the normal male specimens.

In the present study the observations have been on the relative growth of the abdomen. During sex transformation the relative growth coefficient of the female changes and approximate to that of the normal male in the growth coefficient. The slight difference between the transformed female and normal male is not significant.

### CONCLUSION

The observations discussed above indicate that due to the action of the androgenic hormone, the female secondary sexual characters acquire progressively the male form during the post operative intermoult periods, by changes in the allometric coefficient.

### SUMMARY

The coefficients for relative growth differ in the two sexes. The implantation of the androgenic gland into the female transforms into the male, and during this process the relative growth coefficient for the abdomen changes progressively during the post operative intermoult periods. Finally the allometric coefficient assumes nearly the value characteristic for the normal male.

### ACKNOWLEDGMENTS

I am indebted to Prof. R. V. Seshayya, Director, Marine Biological Station, Porto Novo, for suggesting this problem and guidance. My thanks are also due to the University Grants Commission for the award of Research Scholarship to me.

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# STUDIES OF FORMS OF APHELINIDAE (HYMENOPTERA—CHALCIDOIDEA) COLLECTED AT ALIGARH (INDIA)—I

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## INTRODUCTION

ASHMEAD (1904) divided the family *Aphelinidae* into two tribes, viz., *Pteropterycini* and *Aphelinini*, based on the presence of four and five-segmented tarsi respectively. Howard (1907), Mercet (1912 and 1929) and Girault (1915) have followed almost the same system. Compere (1936) in his paper on "The Classification of *Aphelinidae*, however, experienced some

genuine difficulty in adopting Ashmead's division of *Aphelinidae*, since, according to him "the present classification, which places *Casca* Howard in one tribe and *Aspidiotiphagus* Howard in another, because of the difference in the number of tarsal joints, is an artificial one that widely separates these two closely related genera". The present writer has met with a similar difficulty in separating *Marietta* Motsch from *Perissopterus* How, which not only show close relationship with each other, but have even been declared synonyms.

De Santis (1946) took the bold step of replacing the old system of 'tribes' by the 'family' system based on a range of characters, in which he has grouped the various genera under the families—*Calesinae*, *Aphelininae* and *Coccophaginae*. This system, no doubt, is a little complicated, but is decidedly much better in many respects than the older conventional one.

Mercet's (1912) monograph *Los Afelininos* supplemented by his later work (1929), *Los Afelinidos de Espana*, forms a good study of the family *Aphelinidae*. Compere's (1931), 'A revision of the species of *Coccophagus* Westwood', can be taken as reliable work, though it barely deals with even one third the number of recorded genera.

De Santis's (1948) *Estudio Monografico de los Afelinidos de la Republica Argentina*, not only revolutionises the system of classification of *Aphelinidae*, but also contains valuable information on most of the recorded genera.

Compere's paper (1947)—'A new genus and species of *Eurymyioecnema aphelinoides*—*Myioecnema* Ashmead'—has been of service in assigning a place to the genus *Eurymyioecnema* among the *Aphelinidae*. This genus, however, has recently been relegated back to its original family *Elasmidae* by Nikolskaya (1952). Compere's work (1955), 'A systematic study of the genus *Aphytis* Howard—species', is an excellent contribution helpful for the separation of *Aphytis* Howard from *Aphelinus* Dalman and other closely related genera.

Alam (1956), in his paper—"The Taxonomy of Some British Aphelinid Parasites," has utilised some as yet unrecognised, new generic characters, pertaining to the pronotum, the sub genital plate, etc. In this connection, the present writer is glad to be in a position to bring, in the course of his present work, a full confirmation of the taxonomic value of these characters.

In view of the scanty information available on the *Aphelinidae* in India, an examination of a good collection of this group made in the course of the last few years at Aligarh was undertaken and it is the purpose of this paper to record the results of these studies.

TAXONOMIC STUDIES OF APHELINIDAE: DESCRIPTIONS OF NEW SPECIES

A reference to available literature on the group showed that only a few species have been recorded from India and that a good many of the forms in the present collection are new to science. In the course of the work, the type material was compared carefully with that of Dr. Alam's collection and keys for the determination of the different genera and of the various species under each genus were worked out. In view of the widespread occurrence of these tiny wasps parasitising various species of Coccids in India, it is hoped that the present report will prove a good incentive to further work. The present paper is restricted to certain new species under three genera: viz., *Coccophagus*, *Prospaltella* and *Encarsia*, and the remaining forms coming under other genera will be dealt with in the second part, which it is hoped will soon follow. All the type-material has been deposited in the Museum, Zoology Department, Aligarh Muslim University.

*Key to Genera of Aphelinidae—Based on Female Forms*

1. Antennae six- to eight segmented.....2
  - Antennae eleven-segmented; funicle six-segmented including two ring joints; club three-segmented; hind coxae not enlarged; hind tibiae without stiff coarse setae on dorsal margin; scrobes deeply impressed forming an indentation, visible on the head from the dorsal aspect; subgenital plate of uniform width with a wide spherical notch in the middle of posterior margin; outer plates of ovipositor narrow at base, broad and obliquely truncated at apex, dorsal margin broadly inflexed (antennae ten-segmented in males with a two-segmented club).....*Eurymyiocnema* Compere.
2. Antennae six-segmented; funicle three-segmented, third segment longest. .... 3
  - Antennae seven- or eight-segmented.....5
3. Fore-wings hyaline or with a single fuscous patch; legs of uniform coloration; pronotum bilobed with a very narrow median connecting bridge; first valvifer subtriangular.....4
  - Fore-wings with hyaline spots or broad bands of transparent hairs, or body with pronounced white spots or maculations, very frequently with both; legs with one or more dark bands; pronotum broad, of a most uniform width; first valvifer semicircular with articular knobs less prominent; subgenital plate of uniform width

with a wide notch in the middle of posterior margin, a knob on either side of the notch followed by ridges *Marietta* Motsch

- 4 Abdomen with a syntergum, apical dorsal sclerite fused with the modified dorsal remnant of ninth tergum, marginal vein much longer than submarginal vein posterior margin of pronotum concave, subgenital plate bilobed with middle region conspicuously narrow, outer plates of ovipositor with an oblique ridge *Aphytis* Howard

—Abdomen without a syntergum, dorsal apical sclerite separate, marginal vein as long as (sometimes a little longer) or shorter than submarginal vein, posterior margin of pronotum straight, subgenital plate sub-triangular with posterior margin reduced to an angular form with a median notch, outer plates of ovipositor with a submarginal ridge along basal half of the dorsal margin

*Apelinus* Dalman

- 5 Antennae seven segmented 6  
—Antennae eight segmented 7

- 6 Antennae with funicle three segmented, all funicle segments of almost uniform length, middle basitarsus short, stigmal vein ends like a bird's head, ovipositor hardly exerted *Physcus* Howard

—Antennae with funicle four segmented, third segment shortest, club entire, stigmal vein with a swollen and rounded tip, pronotum with anterior and posterior margins deeply concave and a submarginal ridge along each lateral margin, ovipositor exerted by about one half length of abdomen

*Azotus* Howard

- 7 Scape cylindrical 8

—Scape laminated, funicle four segmented, club two segmented, marginal vein clearly much shorter than submarginal vein, stigmal vein somewhat truncated at apex with sensoria in two rows

*Prophyscus* De Santis

- 8 Mesoscutum with a reduced number of setae, less than 18 arranged in bilateral symmetry, posterior margin of pronotum concave in middle

9

—Mesoscutum usually profusely setose, posterior margin of pronotum straight, cilia of marginal fringes of fore wings short, marginal vein clearly more than half as long as the submarginal vein, outer plates of ovipositor with broadly rounded apex, with a broad

marginal inflexion along dorsal margin; flagellum of antennae plainly differentiated into funicle and club.....*Coccophagus* Westwood.

9. Funicle three-segmented, club three-segmented; outer plates of ovipositor narrow at base, truncated at apex with a narrow inflexion along dorsal margin extending up to basal  $\frac{3}{4}$ ths of the plate; subgenital plate with anterior margin straight, posterior margin convex with a median notch and two submarginal oblique ridges on either side of the notch; cilia of marginal fringes of fore-wings long, not exceeding  $\frac{1}{2}$  wing width.....*Prospaltella* Howard.  
—Funicle four-segmented; club two-segmented; outer plate of ovipositor slightly expanded and obliquely truncated at apex with an oblique ridge running along the middle; subgenital plate almost semicircular with anterior margin convex and posterior margin straight with a wide notch in the middle; cilia of marginal fringes of fore-wings not exceeding  $\frac{1}{4}$  wing width.....*Encarsia* Foerster.

# I. New Forms under Genus *Coccophagus* Westwood

*Coccophagus* is one of the most easily recognised genera of the family *Aphelinidae*. Various stable generic characters have been used in this connection by Mercet (1912 and 1930), Compere (1931), De Santis (1948) and Alam (1956). Alam (1956) has, in addition, recorded some interesting new generic characters for *Coccophagus*, viz., those relating to the pronotum and the outer plates of ovipositor, which appear reliable and can safely be taken as stable characters. Compere's (1931) key to species of *Coccophagus* has been amended as below to accommodate four new species herein described.

## *Revised Key to Species of Coccophagus based on Females*

1. Wings smoky.....2  
—Wings hyaline..... 4
2. Pedicel one and one-half times longer than first funicular segment; first funicular segment more than three times as long as wide; infuscated patch on fore-wings interrupted by a diagonal hyaline streak; hind coxae, trochanters, femora, middle portion of metanotum and propodeum yellow; antennae and abdomen black.....*C. rusti* Compere.  
—Pedicel clearly shorter than the first funicular segment, which is about as long as wide; infuscated patch on fore-wings without a hyaline streak:.....3

- 3 Head with yellowish medio frontal line, pedicel slightly longer than wide, postmarginal vein one third of stigmal vein, cilia of marginal fringes of fore-wings one seventh of wing width, submarginal vein with 21 bullae, antennae and body brown *C diaspidis*, sp n

—Head without medio frontal line, pedicel clearly longer than wide, post marginal vein slightly shorter than stigmal vein, cilia of marginal fringes of fore wings one tenth of wing width, sub-marginal vein with 24 bullae, antennae black, body with bluish reflections *C citri*, sp n

- 4 Submarginal vein with 16 bullae, each parapside with 3 or 4 setae, basal one third of abdomen yellow, five cross bands on the rest 5

—Submarginal vein with 14 bullae, each parapside with 5 setae, abdomen with a broad band in the middle

*C javensis* Girault

- 5 Occiput yellow, scape about five times longer than wide, pedicel about one and one half times longer than wide, first funicular segment shorter than the following funicle segments, each parapside with 3 setae, meso postphragma narrowly notched at apex, post marginal vein one half as long as stigmal vein *C manii*, sp n

—Occiput black, scape four and a half times longer than wide, pedicel twice longer than wide, first funicular segment longer than the following segments, each parapside with 4 setae, meso post phragma narrowly rounded at apex with a notch, postmarginal vein one-fourth of stigmal vein *C indicus*, sp n

(i) *Coccophagus manii*, sp n \*

FEMALE

*Head* (Figs 1 A and 1 B)—Orange yellow, wider than long, post-occiput eyes and ocelli blackish, fronto vertex wide, two and a half times wider than long, eyes globular, slightly longer than wide, ocelli in obtuse angled triangle, distance of basal ocelli from occipital margin about one and one-half times the distance from eye rim, cheeks short, much narrower than eye width, antennal sockets placed close to oral margin

*Antennae* (Fig 2)—Orange with brown sensoria, scape long cylindrical about five and a half times longer than wide, pedicel short, over one

\* Named after Dr M. S. Mani, Deputy Director, Zoological Survey of India

and one-half times longer than wide, slightly shorter than first funicular segment; the first funicular shorter than the second and third funiculars individually; second and third segments of equal length and width, about one and a half times longer than wide; club long, three-segmented, more than three times longer than wide, slightly shorter than the funicle; the consecutive segments of funicle-club region with 4, 7, 7, 8, 7 and 4 sensoria respectively.

*Thorax.*—Orange yellow, slightly brownish on the anterior margin of meso-scutum; scutum along the boundaries, axillae, metanotum and propodeum reddish-brown; pronotum small, of uniform width with anterior margin deeply and acutely notched in middle, posterior margin convex; each of the parapsides and axillae with 3 and 2 setae respectively; scutellum broadly rounded at apex; propodeum narrow in middle, expanded on sides; meso-postphragma notched at apex.

*Fore-wings.*—Hyaline, more than twice as long as wide; submarginal vein long, slightly longer than marginal vein, with 12 setae and 16 bullae; marginal vein very long; post-marginal vein very short, about 1/14-th of marginal vein (Fig. 3); stigmal about twice as long as the postmarginal vein; cilia of marginal fringes short, spaced by a distance equal to one-fifth of their length.

*Hind-wings.*—Hyaline, seven times longer than wide; cilia of marginal fringes long, equal to  $\frac{3}{4}$  wing width and spaced by a distance equal to one-fifth of their length.

*Fore-legs.*—Light orange yellow; outer face of coxae with two long setae; apex of femora with a single long seta; basitarsus slightly shorter than the following two segments combined.

*Middle legs.*—Coloration same as on fore-legs; outer face of coxae with three long setae; femora with a single seta near apex; apical rim of tibiae with four pegs; tibial spur shorter than basitarsus; basitarsus as long as the following three segments combined.

*Hind-legs.*—Coloration same as on the fore-legs; basitarsus shorter than the following three segments put together.

*Abdomen.*—Orange yellow with five brownish-black cross-bands on distal two-thirds; longer than thorax; ovipositor concealed; outer plates of ovipositor narrow at base, broad at apex, dorsal margin with a very broad marginal inflexion; second valvifer long; the third element—valvula, oblong, immovably continued with the second valvifer.

*Measurements*—Length of Female 0.717 mm

*Material*—*Holotype* A female specimen bred out on 11-8-1956. Also two female *paratypes* reared on different dates. The species is an endoparasite of *Pseudococcus citri* Risso found on *Citrus medica* L. (*Kagzi limu*) at Aligarh, India.

(ii) *Coccophagus indicus*, sp. n.

Resembles *C. manii* above described but differs from it in the following points

*Head*—Post occiput black

*Antennae* (Fig. 4)—Scape four and a half times longer than wide, pedicel shorter than the following segment, about twice longer than wide, first funicular segment subequal in length but narrower than the following segments, consecutive segments of funicle and club with 6, 6, 7, 7, 6 and 5 sensoria respectively.

*Thorax*—Each parapside with 4 setae, scutellum narrow, narrowly rounded at apex, about as wide as long, meso postphragma narrowly rounded at apex.

*Fore-wings*—Post marginal vein (Fig. 5) very short, about  $\frac{1}{4}$  of stigmal vein.

*Middle legs*—Apex of tibiae without pegs.

*Measurement*—Length of Female 0.83 mm

*Material*—*Holotype* One female bred out on 15-9-1956. Endoparasite of *Pseudococcus citri* Risso on *Citrus medica* L. (*Kagzi limu*) at Aligarh, India.

(iii) *Coccophagus diaspidis*, sp. n.

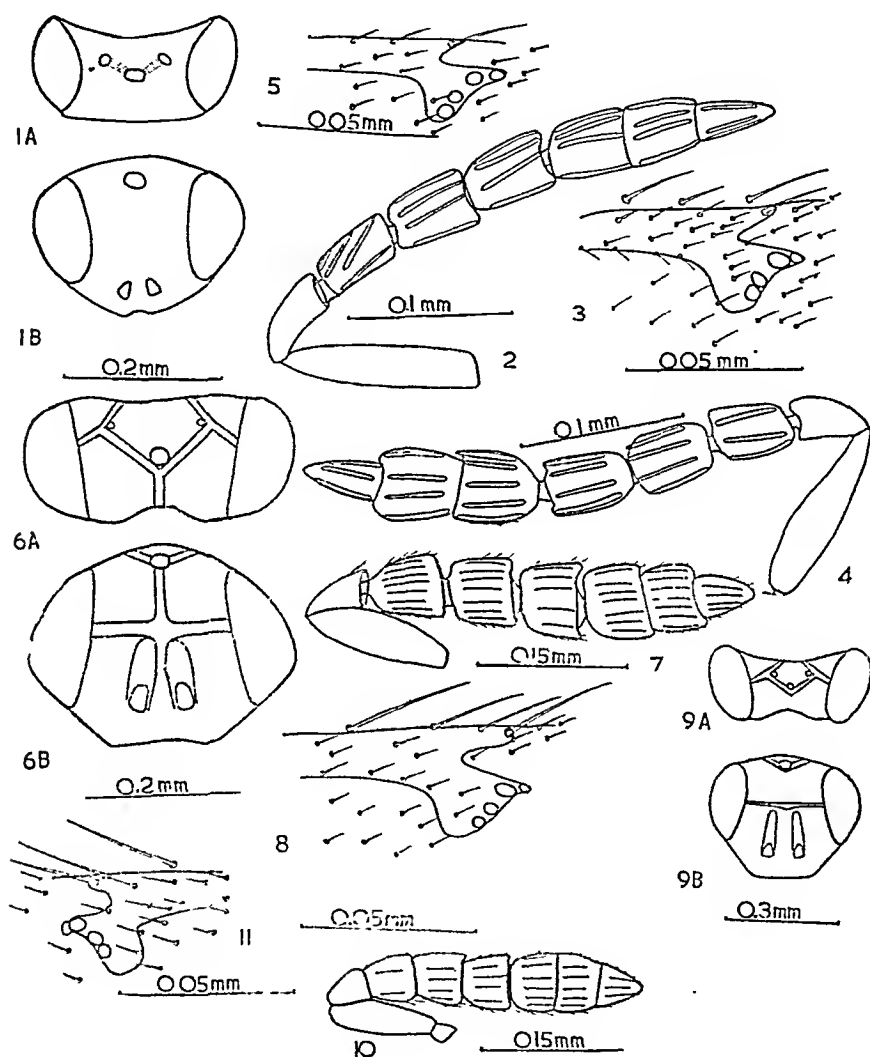
FEMALE

*Head* (Figs. 6A and 6B)—Dark brown with yellowish orbito-occipital, ocellar, medio frontal and facial lines, postocciput, cheeks rusty, head broader than long, fronto vertex horizontal, about twice as wide as long, ocelli in acute angled triangle, basal ocelli removed from eye rim by more than one and one half times their distance from the occipital margin, eyes globular, about as wide as long, cheeks narrower than eye-width, antennal sockets removed by about their longitudinal diameter from the facial margin, scrobes deep, convergent but not meeting above.

*Antennae* (Fig. 7)—Dark brown with an almost white scape; scape very slightly compressed, about three times as long as wide, pedicel slightly



longer than wide, slightly shorter than first funicular segment; first funicular about as wide as long, the second slightly wider than long, the third clearly wider than long; club three-segmented, about two and one-third times



FIGS. 1-11. Figs. 1-3. *Coccophagus manii*, sp.n. Fig. 1. Head vertical view, Fig. 1 B. Head facial view. Fig. 2. Antenna. Fig. 3. Venation of fore-wing. Figs. 4-5. *Coccophagus indicus*, sp.n. Fig. 4. Antenna, Fig. 5. Venation of fore-wing. Figs. 6-8. *Coccophagus diaspidis*, sp.n. Fig. 6 A. Head vertical view. Fig. 6 B. Head facial view. Fig. 7. Antenna. Fig. 8. Venation of fore-wing. Figs. 9-11. *Coccophagus citri*, sp.n. Fig. 9 A. Head vertical view. Fig. 9 B. Head facial view. Fig. 10. Antenna. Fig. 11. Venation of fore-wing.

longer than wide, shorter than funicle, the consecutive segments of funicle-club with 6, 8, 8, 6, 8 and 4 sensoria respectively

*Thorax*—Uniformly coloured dark brown, black on the scuto scutellar suture, pronotum broad, of uniform width with anterior margin notched in middle, posterior margin convex, scutum with 8 rows of setae, parapsides and axillae with 4 and 2 setae each, meso-postphragma narrowly rounded at apex propodeum narrow in the middle

*Fore wings*—Hyaline but infuscated from the marginal vein up to the posterior margin of wing, costal cell narrow, submarginal vein very long, slightly shorter than marginal vein with 7 long setae and 21 hullae, post marginal vein very short,  $\frac{1}{2}$  of stigmal vein, stigmal vein (Fig 8) almost sessile, marginal fringes moderate about  $\frac{1}{7}$  of wing width, cilia spaced by a distance equal to one fourth of their length

*Hind wings*—Hyaline slightly less than 5 times longer than wide, marginal fringes long less than  $\frac{1}{2}$  of wing width, fringe cilia spaced by a distance equal to one fourth of their length

*Fore legs*—Brownish except for tibiae and tarsi which are whitish, apex of femora with a single seta on its inner face

*Middle legs*—Coxae, trochanters, femora excepting apex and base of tibiae brownish, apex of femora and tibiae excepting base and tarsi whitish, tibial spur longer than basitarsus

*Hind legs*—Coxae, trochanters, femora and tibiae dark brown, tarsi light brownish, coxae with three long setae on its outer face

*Abdomen*—Dark brown with inter segmental areas yellowish, sub-oval longer than thorax, ovipositor almost concealed, outer plates narrow at base, broad at apex with dorsal margin inflexed, second valvifer elongated, valvula oblong immovably joined with second valvifer

*Measurement*—Length of Female 0.991 mm

*Material*—*Holotype* One female bred out on 19-9-1959, *Paratypes* Several females bred out on 14-1-1960. An endoparasite on a scale—*Diaspis* sp. occurring on *Saccharum spontaneum* L. (Kans) at Aligarh, India

#### DISCUSSION

*C. diaspidis*, sp. n. runs down in Compere's (1931) key up to *C. rusti* Compere, but differs from it in the following characters

<i>C. rusti</i> Compere	<i>C. diaspidis</i> , sp.n.
1. Antennae black.	Antennae brown.
2. Pedicel one and one-half times longer than wide.	Pedicel slightly longer than wide.
3. Pedicel one and one-half times as long as the first funicular segment.	Pedicel shorter than the first funicular.
4. First funicular slightly more than twice as long as wide.	First funicular almost as as long as wide.
5. Third funicular slightly longer than wide.	Third funicular clearly wider than long.
6. Infuscated patch on fore-wings interrupted by a diagonal hyaline streak.	Infuscated patch on fore-wings not interrupted by hyaline streak.
7. Hind coxae, trochanters and femora yellow.	Hind coxae, trochanters and femora brown.
8. Tarsi brownish.	Tarsi whitish.
9. Median part of metanotum and propodeum yellow.	Metanotum and propodeum brownish.

(iv) *Coccophagus citri*, sp.n.

Resembles *C. diaspidis* sp.n. but differs from it in the following characters:

FEMALE

*Head* (Figs. 9 A and 9 B).—Black with bluish-sheen over frons and face; eyes reddish; medio-frontal line wanting; fronto-vertex less than twice as wide as long; basal ocelli very close to the occipital margin, more than twice this distance from eye-rim.

*Antennae* (Fig. 10).—Black; scape more than three times longer than wide; pedicel clearly longer than wide, much shorter than the following funicular segments; first funicular longer than wide, the second as long as wide, the third wider than long; funicle and club with many sensoria.

*Thorax*.—Black with bluish-sheen over dorsum and on sides; propodeum of uniform width.

*Fore-wings*—Submarginal vein with 5 setae and 24 bullae, post-marginal vein developed, shorter than stigmal vein (Fig 11), marginal fringes short, about one tenth of wing width

*Hind wings*—Wide, less than four times longer than wide, marginal fringes short, less than  $\frac{1}{4}$  of wing width

*Fore legs*—Blackish except for the tibiae and tarsi which are whitish

*Middle legs*—Coxae, trochanters, femora and basal half of tibiae dark brown, apical half of tibiae and tarsal segments white, tibial spur shorter than basitarsus

*Hind legs*—Coxae, trochanters, femora and tibiae dark brown, tarsi white

*Abdomen*—Dark brown

*Measurement*—Length of Female 1.065 mm

*Material*—*Holotype* One female bred out on 20-8-1957 Also kept three female paratypes bred out on different dates Endoparasite on cane mealy bug, *Saccharicoccus* sp on *Sugarcane* at Aligarh, India

## II New Forms under Genus *Prospaltella* Howard

This genus was first described by Howard in 1904 under the name *Prospalta* Ashmead (1904) gave it the present name, since *Prospalta* was preoccupied by a Lepidopterous genus (De Santis, 1948) It is close to *Aspidiotiphagus* Howard, but can be separated from it by the fringes of the fore-wings being narrower than the width of the wing Pronotal characters as indicated by Alam (1956), further help one in their differentiation From *Encarsia* Foerster, to which it is also closely related, it can be separated by the following recently suggested generic characters, viz, subgenital plate with the anterior margin straight, the posterior margin convex, gradually narrowing posteriorly, and without a median notch, outer plate of ovipositor narrow at base broad and truncated at apex with a very narrow inflexion along the dorsal margin, extending up to  $\frac{1}{2}$  of its length

*Prospaltella* seems to be a connecting link between *Coccophagus* and *Aspidiotiphagus* The ratio between the length of the marginal cilia to that of the interspace between them, which is 1.7 in *Prospaltella*, 1.4 in *Coccophagus* and 1.14 in *Aspidiotiphagus*, appears to be sound enough for the separation of these genera

Mercet's (1912) key to the species of *Prospaltella* How has been revised as below to include *P. silwoodensis* Alam and the three new species,

*Revised Key to the Species of Prospaltella Howard based on Females*

1. Fore-wings with indication of post-marginal vein.....2  
 —Fore-wings without indication of post-marginal vein.....4
2. Pedicel twice or more than twice as long as wide; club much longer than the preceding two funicle segments combined; third funicular segment longer than the second.....3  
 —Pedicel as long as wide; club as long as the preceding two funicular segments united; third funicular segment as long as the second; body light yellow; wings hyaline.....*P. lahorensis* How.
3. Body dark brown; antennae dark brown; fore-wings dusky; pedicel more than twice as long as wide, as wide as the first funicular segment; first funicular slightly longer than wide; club longer than pedicel and funicle united; post marginal vein more than one-half of stigmal vein .....*P. silwoodensis* Alam.  
 —Body yellow; antennae light yellow; fore-wings hyaline; pedicel twice as long as wide, wider than first funicular segment; first funicular almost one and one-half times longer than wide; club clearly shorter than pedicel and funicle united; post-marginal vein less than one-half of stigmal vein.....*P. pseudococci*, sp.n.
4. Fore-wings hyaline; antennae uniformly colored orange; marginal vein slightly shorter than submarginal vein.....6  
 —Fore-wings hyaline with infuscated areas; antennae in part brown, dusky or white; marginal vein much shorter than submarginal vein.....5
5. Antennae brownish with the second and the third funicular segments whitish; fore-tibiae with a dusky band; basal two tarsal segments dusky; middle and hind tibiae with two dusky bands; basal tarsal segments dusky; fore-wings infuscated at base and another triangular infuscation with its apex at the stigmal vein and base at the posterior margin of the wing; hind-wings with marginal cilia longer than their width..... *P. murtfeldtii* Howard.  
 —Antennae with first funicular segment and third club segment brown and the rest fuscous; legs uniformly colored light orange with the exception of the basal half of hind coxae which is brown; fore-wings hyaline at base and apex; infuscations at the marginal and stigmal veins extending up to the posterior wing margin; hind

wings with marginal cilia clearly shorter than the wing width  
*P orientalis*, sp n

- 6 Funicular segments of equal length, pedicel narrower than the width of the first funicular *P conjugata* Masi  
 —First funicular segment shortest and least wide, pedicel one and one half times wider than the first funicular *P citri*, sp n

*Key to the Species of Prospaltella Howard based on Males*

- |   |  |                             |
|---|--|-----------------------------|
| 1 | Antennae eight segmented   | 2                           |
|   | —Antennae seven segmented  | 3                           |
| 2 | Funicular segments of uniform length, third segment three times longer than wide           | <i>P conjugata</i> Masi.    |
|   | —Funicular segments increase in length distad, third funicular four times longer than wide | <i>P filicornis</i> Mercet. |
| 3 | Pedicel as long as the first funicular segment   | <i>P lucaspidis</i> Mercet  |
|   | —Pedicel clearly shorter than first funicular segment                                      | <i>P pseudococci</i> , sp n |

(i) *Prospaltella citri*, sp n

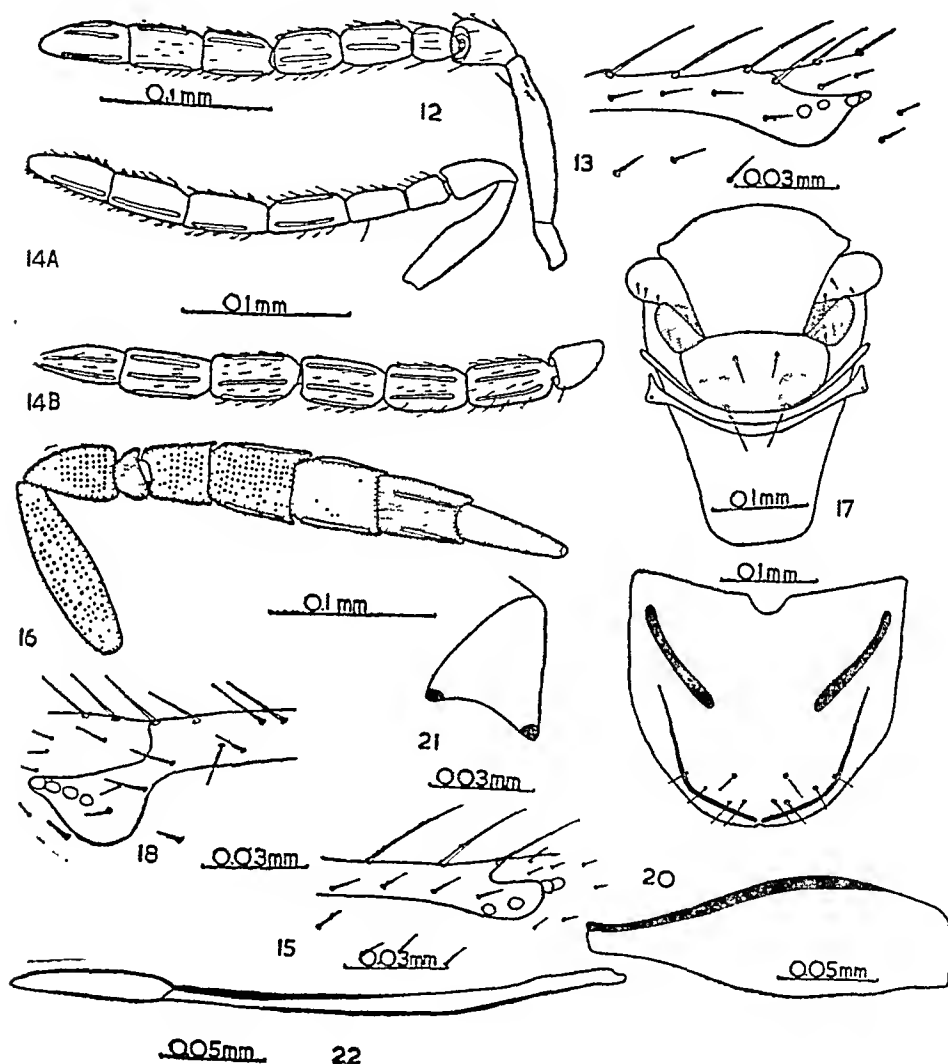
FEMALE

*Head*—Yellowish orange, eyes black, head wider than long, fronto-vertex broad, about twice as wide as long, eyes bare, slightly wider than long, ocelli dark distance of basal ocelli from eye-rim greater than that from occipital margin, cheeks short, almost as broad as the transverse diameter of eye, sub ocular suture distinct and complete, antennae inserted by a length equal to the longitudinal diameter of the antennal socket, scrobes narrow, convergent, mandibles with two teeth and a truncation, maxillary palps two-segmented, labial palps unsegmented

*Antennae* (Fig 12)—Uniformly colored orange, scape nearly five times longer than wide, pedicel short slightly longer than wide, longer than the first funicular segment, the first funicular segment shortest, slightly longer than wide, the second and third segments of almost equal length and width, club three segmented much longer than the funicle, more than five times longer than wide, the second funicular to the third club segments with 2, 2, 3, 3 and 3 sensoria respectively

*Thorax*—Uniformly colored yellowish orange, anterior margin of meso-scutum dark brown, meso-scutum with 8 setae, each parapside and axilla with three and two setae respectively, meso-postphragma narrowly truncated at apex

**Fore-wings.**—Hyaline; two and a half times longer than wide; sub-marginal vein a little longer than marginal vein, with 10 bullae and four long setae; marginal vein very long, thick with seven setae; post-marginal vein wanting (Fig. 13); stigmal vein short; marginal cilia very long, about



FIGS. 12-22. FIGS. 12-13. *Prospaltella citri*, sp.n. FIG. 12. Antenna. FIG. 13. Venation of fore-wing. FIGS. 14-15. *Prospaltella pseudococci*, sp.n. FIG. 14 A. Antenna of female. FIG. 14 B. Antenna of male. FIG. 15. Venation of fore-wing. FIGS. 16-22. *Prospaltella orientalis*, sp.n. FIG. 16. Antenna. FIG. 17. Thoracic dorsum. FIG. 18. Venation of fore-wing. FIG. 19. Subgenital plate. FIG. 20. Outer plate of ovipositor. FIG. 21. First valvifer, FIG. 22. Second valvifer with valvula.

one fourth of wing width and spaced by a distance equal to one-seventh their length

*Hind wings*—Hyaline, cilia of marginal fringes long, more than wing width and spaced by a distance equal to one seventh length of cilia

*Fore legs*—Uniformly colored light yellow, outer face of coxae with two setae near apex

*Middle legs*—Coloration same as on fore legs, apex of tibiae with three short pegs, tibial spur shorter than basitarsus

*Hind legs*—Coloration same as on fore legs, apex of tibiae with three stout setae

*Abdomen*—Yellowish orange, with black patches on sides of second segment and a median black patch on the apical segment, longer than thorax, genitalia concealed, outer plates of ovipositor narrow at base, expanded at apex with dorsal margin thickened, the first valvifer subtriangular with articular knobs slightly prominent, the second valvifer elongated, the third element—the valvula—long, rounded at apex and immovably articulated with second valvifer

Length of female

0.66 mm

*Material*—*Holotype* One female bred out on 10-9-1956 Endoparasite of *Pseudococcus citri* Risso found on *Citrus medica* L. (*Kagzi limu*)

*Prospaltella citri*, sp. n. runs down in Mercet's (1912) key to species up to *P. conjugata* Masi, but differs from it in the following particulars

<i>P. conjugata</i> Masi	<i>P. citri</i> sp. n.
1 Funicle segments cylindrical and of almost equal length	First funicular shortest, second and third of equal length
2 Pedicel as long as first funicular segment	Pedicel clearly longer than the first funicular
3 Consecutive segments of funicle and club with 1, 1, 1, 2, 2 and 2 sensoria respectively	Consecutive segments of funicle-club, with 0, 2, 2, 3, 3 and 3 sensoria respectively
4 Marginal cilia of hind wings shorter than wing width	Marginal cilia of hind-wings longer than wing width
5 Spur of middle tibiae reaching up to one half the length of basitarsus	Spur of middle tibiae clearly exceeding one half the length of basitarsus



(ii) *Prospaltella pseudococci*, sp.n.

FEMALE

*Head*.—Uniformly colored dirty brown with ocelli and eyes black; head wider than long; fronto-vertex three times wider than long; basal ocelli situated at greater distance from eye-rim than from occipital margin.

*Antennae* (Fig. 14 A).—Scape whitish, rest of flagellum yellow; scape fusiform, a little over four times longer than wide ( $0.091 \times 0.023$ ); pedicel twice as long as wide, about twice the length of first funicular segment; first funicular shortest, third longest, second and third segments more than twice as long as wide; club three-segmented, about seven times longer than wide, shorter than pedicel and funicle united; consecutive segments of funicle to club with 0, 1, 2, 3, 2 and 1 sensoria respectively.

*Thorax*.—Uniformly colored rusty; pronotum broad along the sides, narrow in middle; mesoscutum with two rows of two setae each, parapsides with a single seta; propodeum of almost uniform width; meso-postphragma narrowly rounded at apex.

*Fore-wings*.—Hyaline, slightly less than three times as long as wide; submarginal vein slightly shorter than marginal vein with 13 bullae and three setae; post-marginal vein very short, about one-fifth of stigmal vein; stigmal vein developed (Fig. 15), curved and narrowly rounded at apex; cilia of marginal fringes long, spaced by a distance equal to one-seventh their length.

*Hind-wings*.—Hyaline; more than seven times longer than wide, marginal cilia long, clearly longer than wing width, spaced by a distance equal to one-seventh their length.

*Fore-legs*.—Uniformly colored light brown.

*Middle legs*.—Coloration same as on fore-legs; basitarsus more than one and one-half times longer than tibial spur.

*Hind-legs*.—Coloration same as on fore-legs; coxae with two setae on outer face.

*Abdomen*.—Sub-oval, pointed at apex, longer than thorax; brownish at base, apex and on sides, whitish in the middle; genitalia concealed; cercal plates at the apical one-fourth; outer plates of the ovipositor long, narrow at base, broad at apex; second valvifer elongated.

## MALE

Resembles the female except for the following differences

*Antennae* (Fig 14 B)—Pedicel short, about one and one-third times longer than wide, clearly shorter than any of the following segments; funicular segments longer than wide, first longest, twice as long as wide; consecutive segments from the first funicular to the third club with 6, 6, 6, 5, 5 and 2 sensoria respectively

*Fore-wings*—Submarginal vein with 14 bullae and two setae; post-marginal vein absent

*Abdomen*—Six fuscous bands, under surface orange at base and apex; cercal plates at the apex

<i>Measurements</i> —Length of Female	0.648 mm
Length of Male	0.618 mm

*Material*—*Holotype* One female bred out on 28-9-1956 *Allotype*: One male reared out on the same date. An endoparasite of *Phenacoccus iceryoides* Green found on *Rhynchosia mimosae* L.

(iii) *Prospaltella orientalis*, sp.n.

## FEMALE

*Head*—Sculptured, vertex and frons orange; cheeks blackish; ocelli and eyes dark brown, occiput black, oral margin black; wider than long; occipital margin acute, frons depressed, fronto-vertex much wider than long; eyes slightly longer than wide; ocelli in acute-angled triangle, distance of basal ocellus from occipital margin less than half its distance from eye rim; cheeks shorter than eye length; antennae inserted close to the oral margin at a distance of one-eighth of the longitudinal diameter of the antennal socket; scrobes deep; mandibles bidentate with acute teeth; maxillary palps two segmented; labial palps unsegmented

*Antennae* (Fig 16)—Scape excepting apex, pedicel, second and third funicular segments and the first two club segments fuscous; apex of scape, first funicular and third club segments brown; scape long, slightly fusiform, more than three times longer than wide; pedicel about one and three-fourth times longer than wide, as long as first and second funicular segments united; first funicular segment one and one-half times wider than long, second and third funicular segments much longer than wide; club three segmented, four times as long as wide, as long as funicle and pedicel combined; second funicular to third club segments with 1, 2, 2, 4 and 2 sensoria respectively.

*Thorax* (Fig. 17).—Orange with some irregular brownish patches or markings; pronotum, scutum on greater part excepting the anterior margin, base of parapsides, axillae, scutellum on apex, sides and middle brown; metanotum orange in middle, dark brown on sides; propodeum orange; mesopleura brown; metapleura orange; sterna orange; pronotum reticulate, anterior margin with a deep notch in middle, posterior margin almost straight with cup-shaped depressions on sides; metanotum sculptured; axillae subtriangular; propodeum of uniform width; meso-postphragma very broad, truncated at apex.

*Fore-wings*.—Hyaline, infuscation extending from the marginal and stigmal veins up to the posterior margin of the wing; costal cell broad; submarginal vein very long, more than twice as long as marginal vein with 18 bullae and two setae; marginal vein with eight setae; post-marginal vein absent; stigmal vein developed, about one-third of the marginal vein; marginal cilia long, spaced by a distance equal to one-seventh their length (Fig. 18).

*Hind-wings*.—Hyaline, almost five times longer than wide; vein with 13 bullae; marginal cilia very long, less than wing width; spaced by a distance equal to one-seventh their length.

*Fore-legs*.—Uniformly colored light orange; coxae with three setae near apex on its outer face; apex of tibiae with two pegs.

*Middle legs*.—Coloration same as on fore-legs; tibial spur slightly longer than the basitarsus.

*Hind-legs*.—Distal half of coxae dark brown, proximal half of coxae and the remaining part of the leg same as on fore and middle legs; each tarsal segment with a peg near its apex.

*Abdomen*.—Long, narrow at apex; basal one-fourth orange, remaining portion dark brown; subgenital plate (Fig. 19) subtriangular, anterior margin straight, posterior gradually reduced with a notch in the middle; antero-lateral apodemes very prominent; two oblique submarginal ridges extending from the notch; outer plates of the ovipositor (Fig. 20) narrow at base, truncated at the apex with a narrow inflexion along the dorsal margin extending up to the basal three-fourths of the plate; first valvifer (Fig. 21) triangular with concave base, articular knobs not very prominent; second valvifer (Fig. 22) elongated with the valvulae immovably articulated at its apex.

*Measurement*.—Length of Female . . . 0.753 mm. . . .

*Material*—*Holotype* One female reared out on 12-8-1958 *Paratypes* Some females bred on the same date An endoparasite of *Aonidiella orientalis* Newst found on *Eugenia jambolana* L. (Jaman)

*P. orientalis* sp n runs down in Mercet's (1912) key up to species *P. murifeldti* Howard, but differs from it in the following characters

<i>P. murifeldti</i> Howard	<i>P. orientalis</i> sp n
1 Antennae brownish with the third segment whitish	Antennae with the first and second segments brown the rest fuscous
2 Scutum with a brown spot in the middle	Scutum yellowish orange brownish on anterior margin
3 Wings hyaline with infuscated base and another triangular infuscation with its apex at the stigmal vein and base on the posterior wing margin	Wings hyaline with infuscated patch starting at the marginal and stigmal veins and extending up to the posterior margin
4 Hind wings with three long rows of cilia third row interrupted at the posterior third of the wing	Hind wings with two rows of cilia
5 Fore tibiae with an obscure band, first and second tarsal segments dusky	Fore-legs uniformly colored light brown
6 Middle and hind tibiae with two dusky bands, first tarsal segment dusky	Middle and hind tibiae and first tarsal segment uniformly colored orange

### III New Forms under Genus *Encarsia* Foerster

This genus was first described by Foerster in 1878. It is very close to *Prospaltella* Howard but can be distinguished from it by its biarticulate club. Mercet (1912), Compere (1931) and De Santis (1948) also have used this character for separating *Encarsia* from *Prospaltella*. De Santis (*loc cit*) further regarded the heteromerous condition of the tarsi as a generic character for separating the males of *Encarsia* from those of *Prospaltella* where they are pentamerous.

Characters such as (1) the subgenital plate almost circular with the anterior margin convex and the posterior almost straight with a wide notch

in the middle and (2) outer plates of the ovipositor slightly expanded and obliquely truncated at apex with an oblique ridge running in the middle, are suggested at the generic level for its separation from other related genera.

A new species of this genus has also been described by Prasad (1955) from India.

(i) *Encarsia narayanani*, sp.n.\*

FEMALE

*Head* (Fig. 23).—Orange-yellow with ocelli and eyes black, post-occiput dark brown, a broad transverse obscure band across the face touching eyes on either sides and scrobes below; head wider than long; occipital margin rounded; fronto-vertex wide, about one and a quarter times as wide as long; ocelli in an isosceles triangle, basal ocelli close to occipital margin, one and one-half times this distance from eye-rim; cheeks slightly shorter than length of eye; mandibles tridentate; maxillary palps two-segmented, labial palps not segmented.

*Antennae* (Fig. 24).—Inserted close to the oral margin, orange with upper and lower margins of scape brownish, last club segment slightly fumate; scape about five times longer than wide; pedicel wider than funicle, as long as club, about one and one-half times longer than wide; the first funicular segment ring-like, the second shortest, the third, fourth and fifth segments of equal length and width; club biarticulate, four times as long as wide, almost equal to the preceding two segments combined; consecutive segments from the second funicular to last club segment possessing 0, 2, 3, 3, 3 and 2 sensoria respectively.

*Thorax.—Dorsum*: Orange-yellow with pronotum greyish, sutures, outer margins of axillae and expanded portions of propodeum brownish; pronotum (Fig. 25) very narrow in the middle, much expanded on sides, anterior margin deeply and acutely notched in the middle, posterior margin concave; mesonotum with 14-setae, each parapside with 5 setae; metanotum narrow, band-like; propodeum much expanded on sides.

*Fore-wings* (Fig 26 A).—Well-developed, more than two and a half times as long as wide; hyaline, very slightly infumate in the middle from marginal vein down to the post-marginal vein; submarginal vein slightly longer than the marginal vein with 16 bullae and three setae; post-marginal

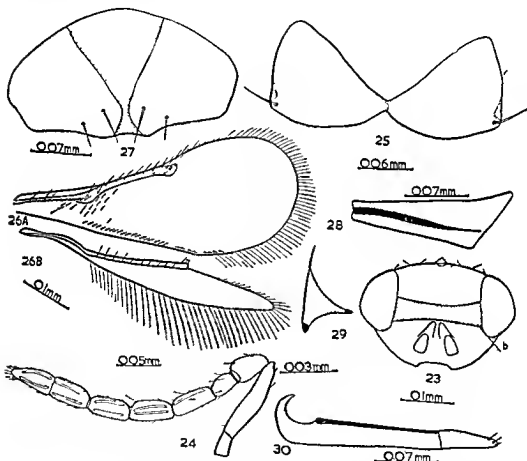
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\* Named after Dr. E. S. Narayanan, Ex. Head of the Entomology Division, I.A.R.I., New Delhi.

vein very short; stigmal vein sessile, rounded at the apex; marginal cilia long, spaced by a distance equal to one-seventh their length

*Hind-wings* (Fig 26 B)—Well-developed, hyaline, more than seven times longer than wide, cilia of marginal fringes longer than the wing width, interspace between cilia equal to one-seventh their length

*Fore-legs*—Uniformly colored whitish, coxae with two setae on outer face



FIGS. 23-30 *Encarsia narayananu*, sp.n. Fig. 23 Head facial view Fig. 24 Antenna. Fig. 25 Pronotum. Fig. 26 A Fore wing. Fig. 26 B Hind wing. Fig. 27 Subgenital plate. Fig. 28 Outer plate of ovipositor Fig. 29 First valvifer Fig. 30 Second valvifer with the valvula.

*Middle-legs*—Coloration same as on fore-legs; tibial spur shorter than basitarsus, apical rim of tibiae with a row of five pegs, basitarsus with three pegs, fourth tarsal segment with a single peg

*Hind-legs.*—Coloration same as on fore-legs; coxae with two setae on outer face; apical rim of tibiae with six stout setae.

*Abdomen.*—Sub-oval, longer than thorax; light orange and fuscous on basal half, brown on sides and apical half; genitalia slightly exserted; subgenital plate (Fig. 27) almost semicircular with anterior margin convex and posterior margin almost straight with a wide notch in middle; outer plates of ovipositor (Fig. 28) slightly expanded and obliquely truncated at apex with an oblique ridge running in the middle of the plate; first valvifer (Fig. 29) subtriangular with articular knobs little prominent; second valvifer (Fig. 30) of almost uniform width with dorsal margin thickened throughout, the valvula long, rounded at apex and immovably continuous with the second valvifer.

*Measurement.*—Length of Female .. 0.703 mm.

*Material.*—*Holotype*: One female bred out on 28-8-1956. An endoparasite of *Phenacoccus iceryoides* Green on *Hibiscus rosasinensis* L. (Gurhal).

*E. narayanani*, sp.n. runs down up to *E. elegans* Masi in Mercet's (1912) key, but differs from it by the possession of a pedicel much longer and wider than the first funicular segment and of a ring-segment situated between the pedicel and the funicle.

#### ACKNOWLEDGMENTS

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# BREEDING RICE VARIETIES RESISTANT TO BLAST DISEASE CAUSED BY *PIRICULARIA ORYZAE* CAV.

## II. Selection of Resistant Varieties of Early Duration from the Genetic Stock\*

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### INTRODUCTION

ONE of the principal lines of investigations undertaken at the Central Rice Research Institute, Cuttack, is breeding for blast resistance through isolation of resistant varieties from the Genetic Stocks. As there are nearly 4,000 rice varieties maintained in the Genetic Stocks at the Institute, for convenience of handling, the varieties are taken up in lots of about 500 varieties each and tested and classified into various groups of relative susceptibility. The varieties selected as resistant and moderately resistant in such tests are sent to the State Departments of Agriculture for trials of their performance under local conditions (Kulkarni, 1959; Venkatakrishnayya and Delvi, 1960; Mathur and Misra, 1961).

The results of the tests carried out during 1948 to 1954 with a first lot of 470 types from the Genetic Stock have been presented earlier (Padmanabhan and Ganguly, 1959). These varieties were departmentally released types from different Rice Research Stations in India and abroad. In the tests, five varieties, viz., Bj-1, Co-4, S-67, SM-6 and SM-9 were classified as resistant and sixteen varieties, viz., ADT-12, AKP-8, AKP-9, AS-2, BAM-4, CH-55, Co-25, Co-26, CP-6, CP-9, H-755, MTU-5, Mugad-249, PTB-10, S-624 and SM-8 were classified as moderately resistant to blast.

In the present contribution results are presented of such tests carried out from 1954 to 1961 with a group of early duration varieties in the Genetic Stocks, maturing within 120 days under Cuttack conditions (21° N.L.).

### MATERIAL AND METHODS

There are 490 varieties of early duration (maturing within 120 days) available in the Genetic Stocks, a representative collection of rice accessions from India and other parts of the world.

\* Part I of the series appeared in *Proc. Ind. Acad. Sci.*, 1959, 50 B, 289-304.

The material was rapidly screened under artificial inoculation at the seedling stage during the first three years in both the main and the second crop season (*viz.*, in August and February, respectively). The techniques adopted for bringing about artificial infection, scoring the infection, and classifying the types into different susceptibility groups were the same as evolved and reported in the previous communication in 1959 (Padmanabhan and Ganguly, *loc cit*)

Besides artificial inoculation tests, all the types were observed for their reaction to natural infection of blast at seedling, post planting, and flowering stages in the maintenance plots, and the susceptible types were progressively eliminated accordingly. The varieties which emerged as resistant in the screening tests in seedling stage were carried forward to the field tests

### Field Tests

The field tests were carried out for three successive years with the varieties carried forward from the screening tests

In the seed-beds, the test varieties were again exposed to artificial infection; a week before transplanting, all the beds were covered with wet coarse cloth curtains on sturdy wooden framework to form 5' x 8' x 6' high humid chambers. Spore suspension, prepared from a culture of the fungus (Cuttaek isolate) in oat-meal agar containing thiamine (0.25 mg in 1,000 ml) and biotin (0.015 mg in 1,000 ml) and incubated at about 25° C for 7-12 days, was used for the artificial infection of seedling in the beds. Artificial infection was carried out for three successive days, late in the evening. The reaction of the varieties was noted after one week.

As has been stated earlier, heavy blast incidence could be induced in the field by late planting (after the middle of August) under adequate fertilization—60 to 80 lb N/acre. In addition, the test varieties were flanked on either side with Co-13, a highly susceptible variety. The blast infection which developed severely on Co-13 constituted a heavy source of inoculum for the varieties under test.

### RESULTS

Out of 490 varieties of early duration under tests, 149 varieties had been already tested and found susceptible in the first phase of this programme, *viz.*, in the years 1948-1953. The remaining 341 types are classified as shown in Table I.

TABLE I  
*Number of varieties tested and their classification in regard to  
 their susceptibility to blast*

Kind of tests	Number of types moderately susceptible to susceptible	Number of types moderately resistant	Number of types resistant	Number of types unclassified
1. In artificial inoculation test (1954 to 1958) seedling stage .. ..	214	..	..	..
2. Under field tests (1959 to 1961) (both foliar and neck-infection) ..	23	8	4	92
	237	8	4	92*

\* These 92 varieties out of 341 types could not be classified, for the same were not tested sufficiently and, therefore, require to be tested in the next such series. The final reaction of these 92 types will be given in a subsequent communication.

The varieties finally selected as resistant and moderately resistant to blast are mentioned in Table II.

The list of varieties, classified into different susceptibility groups, is presented in Table III.

## DISCUSSION

Due to the rigorous criteria adopted, and the elaborate procedure followed in carrying out the tests, only nine varieties, four in the present tests and five in the earlier series, could finally be selected as resistant to blast disease of rice out of more than one thousand varieties studied. As has been discussed in detail in the first communication, the reaction of a variety to both leaf and neck-infection has been taken into consideration in selecting for resistance. Amongst the varieties which emerged as resistant in the seedling stage tests, some continued as resistant to leaf-infection in the field, but showed various degrees of susceptibility to neck-infection. The opposite phenomenon, viz., susceptibility to leaf-infection but resistance to neck-infection has also been met with by the senior author in other studies conducted with varieties drawn from all parts of the world.

TABLE II

*List of varieties selected finally as resistant or moderately resistant to blast disease of rice*

Sl No	AC No	Type No	Name of variety	Place of origin	Reaction
1	27	CH-27	Chang Ming Yunan	China	MR
2	28	CH-28	Si Kong-Ya an (Red husk)	China	R
3	71	CH 71	Kaoshiung No 18	China	MR
4	533	Jap-7	Aichi Asahi	Japan	R
5	1613	T-1446	Surlu Black	S Canara (India)	R
6	1771	T-1715		Belgian Congo	MR
7	1966	T-2009		UP (India)	MR
8	2082		Seluz 44	Australia	R
9	2250	T 6522		Coimbatore (India)	MR
10	2489		Nep-vai	Indo-China	MR
11	2565	T-1026		Coimbatore (India)	MR
12	2597	T-1160		Coimbatore (India)	MR

R—Resistant, MR—Moderately resistant, AC—Accession number

Templeton *et al* (1961), finding that there was a close correlation between seedling reaction and neck rot in twelve varieties which they studied, have been led to the conclusion that testing for resistance could be based upon seedling reaction alone. Since such a correlation is more common than variability, the latter phenomenon may not occur when the reactions of a limited number of varieties only are taken into consideration.

Further work, done in Japan (R. Ito, 1963, International Symposium on Blast Disease of Rice, the Philippines) and in Formosa (Taiwan) by Hashuoka (1950), also shows that rice varieties do not react always in the same manner to leaf and to neck-infection. Therefore, it is very necessary to test

TABLE III  
Reaction of rice varieties of early duration (120 days) of Genetic Stock  
to blast disease at C.R.R.I., Cuttack, during 1954-1961.

Place of origin	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible
1. Andhra Pradesh	..	..	T. 1150 (Nellore), Nalla (Thakavadi), Thatta- kottavadi	SLO 16 (Kasipichodi), H.R. 67, H.R. 21, T. 894, Mottakar
2. Assam	..	..	T. 2058 (Assam 21), T. 2063 (Bengal 5), T. 2072, T. 2085	T. 623 (Tepi Dumai D. 138/2), T. 626, T. 639 (Lundumra), T. 2056 (Assam 19), T. 2057/ 1 (Assam 20/1), T. T, B, Cross 313-11, C. 203-3
3. Bengal	..	..	T. 657 (Patnai—Gosaba T. 230)	..
4. Bihar	..	..	BR 17	T. 619 (Kolaba strain), T. 922, T. 924 (Jana paddy), Koda
5. Bombay	..	..	Patnai-6, Waner-1	68-1, Khare Bhat
6. Kashmir	..	..	It. 8	Aust. 370, Japani 1138
7. Kerala	..	..	T. 239 (Chamban), T. 240 (Salem Chamban), T. 247 (Kasturi Kazhima), T. 1357, T. 1457 (Mun- daga Champan), T. 1452	T. 10 (Kochuvithu), T. 246 (Arupatham), T. 249 (Potta Modan), T. 251 (Navara- nethi), T. 253 (Potta chornal), T. 1344 (Chuvannvathan, modan), T. 1351, PTB, 28 (Katta- modan), T. 1829, T. 2012, T. 2015.

TABLE III (Contd)

Place of origin	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible
8 Madhya Pradesh			Safeda (Kota)	T 23 (Safeda), T 1687 (Surmatia), T 1690 (Ajan), Safed Dhan, R <sub>2</sub> (Nungi No 17 No 13, Central Farm, Ujan
9 Madras	AC 2565 (T 1026), AC 2597 (T 1160), AC 2250 (T 6522)	T 499, T 522 (Vellai kuruvai), T 1198, T 1213 (Pavitrani samba) T 1704, T 2107, T 2108, Rascadam, Uvar kondan, T 991	T 115 (Extracted at Coimbatore), T 418 (Arupathum Kuruvai) T 480 (Vari samba), T 482 (Panamara samba), T 181 (Arupathum vellai), T 182 (Sornavari), T 186 (Thathan samba), T 127 (Early Kuruvai), T 257 (Avasara samba), T 385 (Gorkondaro), T 399 (Sarapalli samba), T 407 (Malayalatham samba), T 487 (Chitrakali), T 504 (Mapilai samba), T 508 (Jhooyare), T 525 (Veral samba), T 921 (Arubatham samba), T 1200 (Kuthalai), T 1155 (Vaddan samba), T 1274, T 1483 (Salem No 3) T 1488 (Anaikomban), T 1494, T 1498 (Kattai samba), T 1507 (Sanna vathan), T 1738 (Swarna-	

10.	Mysore	AC. 1613 (T. 1446-Surli Black)	..	..	vari), T. 1770, T.K.M. 3 (Sornavari), T. 960 (Extracted), T. 1230, Thellathokka vadlu, ASD 8, ASD 9 T. 18 (Kaptasambatha), T. 25 (Gangasalabatha), T. 292 (Sirang), T. 690 (Kemburja), T. 1151
11.	Orissa, C.R.R.I.	..	..	T. 1278, T. 1516, T. 1525, T. 1519, T. 1727/1, N×60 (Beali) Natural cross T. 647 (Jhona), T. 882, T. 883	
12.	Punjab	..	..	H. 287/1 (Nagina), N. T. 2018, T. 2019, T. 2024, Ch. 2, N. Ch 11, No. Ch 1	
13.	Uttar Pradesh	..	AC. 1966 (T. 2009)	hsn, H. 262/1 S. 78 (Gajaur), S. 112 (Mutri), S. 114 (Bhanli red), S. 120 (Bhanli), H. 64	
14.	Australlia	AC. 2082 (Seluz 44)	..	..	
15.	Belgian Congo	..	A. 1771 (T. 1715)	T. 1718	
16.	Brazil	..	..	T. 1654 (Cotetas)	
17.	Burma	..	..	T. 282 (Mo Myawtol), T. 337, T. 2992	
18.	Ceylon	..	AC. 27 (Ch. 27)	E.K. 70 (Raja Mundri)	
19.	China	..	(Chang Ming Yunan)	Ch. 79 (Taiwan variety), T. 956, T. 957, T. 968, T. 969, T. 971, T. 974, T. 976, T. 978/1 (Igangmia) Yang Tsaw), T. 989, T. 979, T. 980, T. 987, T. 990, T. 1002, T. 1003, T. 988, T. 993, T. 999, T. 1000,	

TABLE I (Contd)

Place of origin	Resistant	Moderately Resistant	Moderately Susceptible	Susceptible
20 French West Africa (Niger Segon)	(Sikong Ya an Red husk), AC 71 (Ch 71) (Kaoshnung No 18)	T 1009, T 1010, T 1020, T 1023, T 1030, T 2112, T 2114, T 2115, T 2116 (Ning Sai Sia Tao), T 2120 (Shengrdt Seiro), T 2121 (Kwei chow 28), T 2125	T 1004, T 1006, T 1007 (Siao Pa-Kuo), T 1011, T 1018, T 1021, T 1027, T 1028, T 1031, T 1032, T 1033, T 1038, T 1042, T 2111, T 2118, T 2122 (Tsan Hus 4)	
21 Indo-China		T 697 (Sikaso)		
22 Iraq (Consul)		AC 2489 (Nep vai)		
23 Italy		T 1815		T 1820 (Nauma)
24 Japan	AC 533 Jap 7 (Aichi Asahi)	T 730 Early wright Jap 4 (Aikoku), Jap 8, (Norn I), T 1985 (Japan 11)	T 1665, T 1680, T 1682 T 1979 (Japan 3), T 1980 (Japan 4), T 1986 (Jap 12), T 1993 (Jap 19)	
25 Nigeria			Farin Iri	
26 Portugal		EAN No 6	Precoce-6, Chines, Ardizzone, EAN No 4	
27 Russia		T 911/1	T 899	
28 South Africa			DS I	
29 USA		T 1158, Canulla (Communo)	USA I (C1 1645), T 727 Magnolia	



for both leaf- and neck-resistance to blast before a variety can be turned as "resistant" and released for cultivation.

### SUMMARY

1. Screening tests were carried out with 490 early duration varieties to isolate varieties resistant to blast disease of rice.

2. On the basis of the reaction of the varieties in the seedlings, post-transplanting and neck-infection stage, four varieties have been selected finally as resistant and eight as moderately resistant to blast disease of rice.

### ACKNOWLEDGEMENTS

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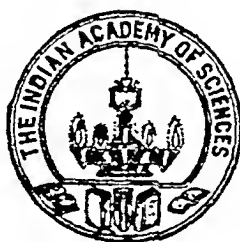
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# MORPHOLOGICAL, HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE PITUITARY GLAND OF *CIRRHINA* *MRIGALA* (HAMILTON)

BY BACHAN LAL\*

(Central Inland Fisheries Research Institute, Barrackpore)

Received October 3, 1963

(Communicated by Dr. B. S. Bhimachar, F.A.S.C.)

## INTRODUCTION

THE morphology and histology of piscine pituitary has been reported from time to time but much less as compared to the voluminous work on mammals, birds, reptiles and amphibians (Pickford and Atz, 1957). While the morphology and histology of the fish pituitary has engaged the attention of workers like Herring (1908, 1913), Tilney (1911), Stendell (1914), deBeer (1923, 1926), Mathews (1936, 1939), Charipper (1937), Bell (1938), Scruggs (1939, 1951), Kerr (1942 *a, b*, 1943, 1948), Atz (1953), Olivereau (1954), Sathyanesan (1958, 1960 *a, b*), Sundararaj (1959), Robertson and Wexler (1962 *a, b*), the study of the histophysiology of the gland in spite of its importance is still in its infancy. The paucity of a thorough knowledge of this gland has always been a hurdle in understanding the reproductive physiology of fish. The interrelationship between the pituitary and gonads has been well established since the work of Houssay (1931), Cardoso (1934), Pereira and Cardoso (1934), Ihering (1937), Ihering and Azevedo (1934). The observations made by the above authors have given great impetus to the experimental work on the control exercised by pituitary over the gonadal development and also on the use of the pituitary hormone as a ripening agent to induce spawning or spawning behaviour in fish. Chaudhuri (1960) and Alikunhi *et al.* (1960) have successfully induced the Indian major carps to breed in captivity with the help of pituitary hormone injections.

Very little attention has been paid to the histology and histophysiology of pituitary of the Indian major carps, which are the most important species used in inland fish-culture. Practically no information is available on the

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histophysiological factors responsible for inhibiting the breeding of Indian major carps in impounded waters Khan (1962) briefly describes the morphology and histology of the pituitary of *Labeo rohita* and attempts to draw some conclusions on the functional anatomy of the gland The investigations reported in this account have hence been taken up with a view to gain insight into the seasonal changes that take place in the cellular composition of pituitary of one of the Indian major carps, *Cirrhina mrigala* (Ham), to assist a better understanding of the probable histophysiological factors responsible for the release and inhibition of the spawning behaviour in lotic and lentic environments respectively

### MATERIAL AND METHODS

The samples of the pituitaries were collected from the fish of different maturity stages from the Vaisle river (30 miles from Gwalior) To remove the pituitary, the dorsal portion of the brain was exposed in a live fish by a deep incision removing the tissues and the bones The brain was later removed with a spatula The pituitary gland thus exposed was removed by means of a fine pair of forceps and immediately transferred to various fixatives With a little practice the entire operation could be completed in a couple of minutes Paraffin sections were prepared from the material fixed in Bouin's picroformol, Zenker-formol, Helly's fluid, neutral formalin, 80% alcohol and Carnoy's fluid for various histological and histochemical tests Gelatin sections of the material fixed in formol calcium (Baker, 1946) with or without postchroming were studied for lipid and carbohydrate tests Anderson's modification of Mallory's triple stain, Heidenhain azan, Mallory's triple stain, Mallory-Heidenhain and Foot's modification of Masson's trichrome techniques were used to distinguish the acidophils from the basophils Periodic acid schiff test (McManus, 1946) and aldehyde-fuchsin (AF) method of Gomori (1950) as well as its modification (Halmi, 1952) with Lugol's iodine and/or potassium permanganate oxidation to distinguish thyrotrophs from gonadotrophs were also conducted

The following were the histochemical methods employed during the present investigations —

*For lipids* — (1) Sudan black B alcoholic modification (Baker, 1949) Some sections were also stained with other lipid colourants as described in the text

(2) Acid haematin test (Baker, 1946) with pyridine extraction control method for phospholipids



*For carbohydrates.*—Periodic acid-schiff test (McManus, 1946; Hotchkiss, 1948) as given by Pearse (1960) with the acetylation technique (Lillie, 1954) was carried out on formol-calcium-fixed gelatine sections as well as on Bouin-fixed paraffin sections.

*For amino-acids.*—(1) Bensley and Gersh modification of Millon reaction (Pearse, 1950) for tyrosine.

(2) Baker's modification of Millon reaction (Pearse, 1960) for tyrosine.

(3) Ninhydrin-schiff method for protein-bound  $\text{NH}_2$  groups (Yasuma and Itchikawa, 1953).

*For nucleic acids.*—(1) Feulgen reaction (Pearse, 1960) for DNA.

(2) Methyl-green/pyronin and ribonuclease method for RNA (Brachet, 1942).

(3) Methyl-green/pyronin Y method for RNA and DNA (Kurnick, 1955).

(4) Gallocyanin-chromalum method for nucleic acid (Einarson, 1951).

(5) Extraction methods for nucleic acids (Casselmann, 1959; Pearse, 1960).

## OBSERVATIONS

*General morphology and orientation of the pituitary and its parts.*—The pituitary of *Cirrhina mrigala* (Hamilton) is spherical in shape in smaller individuals becoming approximately pear-shaped in adults (Plate XII, A). It is situated ventral to the brain enclosed in a saddle-shaped excavation of the cranial floor—the sella turcica. Such a cavity has been reported in a few fish only and this condition approaches that associated with mammalian pituitary. This is a compact gland covered dorsally and ventrally by connective tissue membranes. The floor of the infundibulum is produced into a cone-shaped structure representing a short and thick infundibular stalk. Thus it is of lepto-basic type according to Bretschneider and Duyvené de Wit's (1947) nomenclature. The infundibular stalk pierces through the connective tissue membrane and is attached to the pituitary in the mid-dorsal region, i.e., dorso-basic type. Due to the presence of protective membranes it has been possible to remove the gland alone, without any injury.

The pituitary of *Cirrhina mrigala* is composed of nervous and glandular components known as neurohypophysis and adenohypophysis (pars glandularis) respectively. The neurohypophysis which is also called as pars nervosa sends its branches into the glandular components of the adenohypophysis.

The adenohypophysis consists of three well defined regions, viz, pro, meso- and meta adenohypophysis (Pickford and Atz, 1957). The meta adenohypophysis is demarcated from pro and meso adenohypophysis by a deep suture (Plate XII, B). There is no such morphological differentiation between pro and meso adenohypophysis but the two regions can, however, be distinguished histologically. The three regions are orientated along a lineo-vertical (anterio-ventral) axis (Plate XII, B) as in trout and salmon (Robertson and Wexler 1962 a b) and in *Callichrous pabda* (Singh and Sathyanesan, 1962). The pro adenohypophysis which is the smallest region is situated anteriorly capping a portion of the meso adenohypophysis which lies posterior to the pro-adenohypophysis but also extends to its dorsal and ventral extremities. The meta adenohypophysis lies ventral to the other two regions.

### HISTOLOGY OF THE PITUITARY

The sagittal sections of the pituitary reveal four histologically distinct parts viz, pro, meso and meta adenohypophysis and neurohypophysis (Plate XII, B). A significant feature is the absence of hypophysial and infundibular cavities in the pituitary proper.

**Pro-adenohypophysis**—This is the smallest part of the hypophysis and is anterior in position. The cells mainly take the orange G or azocarmine stain depending on the time of differentiation, fixation and the technique used and are thus acidophils (Levenstein, 1939, Scruggs, 1939). A few cells were found to take aniline blue stain showing thereby that this region does not possess many basophil cells. However, chromophores are found scattered here and there. These look like naked nuclei due to the scanty non granular cytoplasm and ill-defined cell limits. The cells vary in size between six and eight micra in diameter and show centrally placed nuclei revealing the chromatin granules in some preparations. These cells are periodic acid schiff (PAS) and aldehyde fuchsin (AF) negative. AF method, if performed after potassium permanganate oxidation, stains neurohypophysial branches deep blue, thus rendering its distributional study very easy. The cell columns of the ventral region of the pro adenohypophysis are separated by the basophil cells of the meso-adenohypophysis (Plate XIII, A). These basophil cells apparently appear to belong to pro-adenohypophysis but actually they are the cells of meso adenohypophysis which probably did not segregate completely during the development of the gland (Scruggs, 1951). These cells are stained with AF and PAS techniques and reveal colloid bodies ('coagulated contents of the vacuoles') inside the cells which are of different sizes and vary in number. No 'lilac basophil' cells are present.

*Meso-adenohypophysis.*—The meso-adenohypophysis is made up almost entirely of interspersed masses of large and deeply staining basophil and acidophil cells. The acidophils which average between six and eight micra in diameter are of two types, viz., fuchsinophil and orange G cells. The basophils are of various sizes and shapes ranging from a small round cell to those irregularly shaped and measuring from sixteen to twenty micra in diameter. The nuclei of small cells are generally round and lie in the middle but in larger cells they are found to be on one side thus causing a bulging of the cell membrane. The nuclei are rarely stained. The granulation of these cells varies at different maturity stages. Immediately before spawning, each cell shows maximum granulation and intense colouration. However, some of them may show less colouration—probably due to degranulation (Sundararaj, 1959). The large vacuoles are easily discernible (Plate XIII, D, F). In the present study the basophil cells could not be differentiated into the two main functional cell types—the gonadotrophs and thyrotrophs by the application of PAS and AF methods as reported by Atz (1953). These cells, however, could be distinguished into two types, one with colloid-bodies and the other without. The peripheral cells reveal globules of different sizes which are stained with PAS (Plate XIII, C). The largest of these globules measures up to twelve micra in diameter. These globules may either be found in the cells directed towards the branches of the neurohypophysis (Plate XIII, C) or may be intercellular also (Plate XIII, E). However, they were not located inside the neurohypophysial branches. They increase in number and size with the advancement of maturity and probably reach the maximum just after spawning (Plate XIII, E). Mostly the colloid-globules in the peripheral cells are acidophilic but some of them may be basophilic or even show duplex nature, i.e., the medullary region is made up of acidophilic material with a basophilic cortex (Plate XII, F). Each of the basophilic cell present in the central region of the meso-adenohypophysis may reveal a colloid-globule which may be completely acidophilic or basophilic. These globules which are minimum in number and size in the first and second stages of maturity increase in size and number as maturity advances, finally attaining maximum size and number in the fifth and sixth stages of maturity (Lal, 1962). The pituitaries collected from the spent fish reveal the vacuoles in the central basophilic cells in place of colloid-bodies, thus suggesting that the latter possibly play some role in initiating the spawning behaviour or spawning act in the fish. No colloid-bodies are present in the acidophil cells. The chromophobes are few and interspersed among the basophils or acidophils. The blood supply is represented by capillaries as well as by large blood sinusoids

(Plate XIII, F) The increase in number of basophils and their vacuolization is found to be directly proportional to the maturation of the gonads

*Meta-adenohypophysis*—The meta-adenohypophysis receives the main trunk of neurohypophysis which branches extensively (Plate XII, B, C) in this glandular region so much so that it occupies more than half of the volume in this region, and is closely knit with the nervous matter. This portion consists mainly of acidophil cells. This condition which has been reported in a few fish is discussed in detail elsewhere in this account. The cytoplasm of these cells contains fine granules taking red or orange colour, depending upon the technique and time of differentiation. The vacuoles are conspicuous by their absence in all the stages. The chromophobes, as in other regions, are discernible here and there but their number is comparatively less. The 'amphiphils' are absent. The basophils which have always been reported as being either the sole or main constituents of this region as reported in *Fundulus* sp (Mathews, 1936) and in *Mystus seenghala* (Sathyanesan, 1960 a) are absent in the present case.

The acidophil cells measuring approximately between six and eight micra in diameter may be distinguished into two types with the help of Mallory-Heidenhain and Mallory triple stains. The central cells take the fuchsophil colour, whereas the distal cells, i.e., those opposite to the meso adenohypophysis, take the orange colour. The nuclei of acidophil cells are placed on one side rather than in the centre. However, they are spherical or oval in form. The nuclei reveal the presence of small chromatin granules. The two types of cells are almost similar in structure. They may be present in groups or in cords arranged along the neurohypophysial branches separated by thin sheaths of connective tissue. The cells are mostly produced into cone-shaped structures when their apices face the neurohypophysial branches. Small blood capillaries containing red blood corpuscles are associated everywhere with the neurohypophysial branches in this region of the carp's pituitary (Plate XII, E).

The chromophobes are also present among the acidophils with large vesicular, centrally placed nuclei containing the nucleoli. The chromatin granules are also revealed in some preparations. But as the cytoplasm is very scanty and non-granular, they look like naked nuclei. The cell walls are not very distinct. The acidophil cells are PAS-negative. The neurohypophysial branches are clearly revealed with aldehyde fuchsin (AF) technique of Gomori (1950) when used after potassium permanganate oxidation.

*Neurohypophysis (pars nervosa)*—The infundibulum is continued into the adenohypophysis (pars glandularis) as neurohypophysis. It sends small

branches to the pro- and meso-adenohypophysis. The main trunk passes downward through the middle of the meso-adenohypophysis—dividing the latter into two halves (Plate XII, D) and enters the meta-adenohypophysis where it arborizes extensively as shown in Plate XII, C. Thus all the three glandular regions are well innervated by the ramifications of the neurohypophysis.

The neurohypophysial portion passing through the middle of the meso-adenohypophysis reveals thick nerve fibres which may be straight or wavy extending lengthwise along the main trunk (Plate XII, D). It contains the nuclei of neuroglia cells as found in other fishes. Large blood vessels containing red blood corpuscles are also present in the main trunk of the neurohypophysis (Plate XIII, B) as well as along the branches supplying the hypophysis. The presence of large blood vessels in the neurohypophysis between meso- and meta-adenohypophysial region as shown in Plate XIII, B suggests that the blood supply enters the pars nervosa near the cleft between meso- and meta-adenohypophysis. Such an observation has also been reported in case of *Cyprinus carpio* (Scruggs, 1951). The meta-adenohypophysis is extensively supplied with blood capillaries—a rare observation which will be discussed later on.

The neurohypophysial distribution is clearly revealed by AF method of Gomori (1950) and its modification by Halmi (1952) after potassium permanganate oxidation. With this technique the various branches of the neurohypophysis containing small bodies of different sizes, coloured deep-violet, can be easily made out. These bodies could not be made out by any other technique adopted during the present investigations. The thin connective tissue sheath limiting the neurohypophysial branches are moderately coloured with PAS technique of McManus (1946).

#### HISTOCHEMICAL OBSERVATIONS

*Carbohydrates.*—The basophil cells which are predominant in the meso-adenohypophysial region are coloured brilliantly with periodic acid-schiff (PAS) technique after McManus (1946) and AF method of Gomori (1950) with potassium permanganate oxidation both in the gelatin sections of the formol-calcium-fixed post-chromed material as well as in the paraffin sections of the material treated with various fixatives. The strong PAS-positive reaction of granules scattered throughout the cytoplasm and the globular bodies (colloidal material) inside the basophil cells indicate their carbohydrate nature. The globules appear more intense (deeply stained) than the granules. This colouration of the cytoplasmic granules and the globular bodies of the basophil cells with PAS is not diminished after the treatment with any of the lipid solvents such as cold or hot acetone or hot alcohol or methanol/chloroform,

etc. This together with their negative reaction to the lipid colourants (*vide infra*) shows that these cytoplasmic inclusions are composed of or contain some carbohydrate material, and their positive reaction in PAS is not due to the presence of any lipids. This point is further confirmed by the acetylation technique of PAS reaction (Lillie, 1954). The control sections, which were treated with a mixture of pyridine and acetic anhydride, always gave a completely negative reaction or, at least, diminished to a great extent in Schiff's reagent, but their colouration was restored after treatment with 20% ammonia in 70% alcohol at 37° C for 24 hours, whereas in the sections treated with 0.1 N-KOH the restoration of the colour was incomplete and did not give uniform results. It may be pointed out here that the colouration of the globular bodies after acetylation technique is not restored whatever the reversal technique followed. Thus, according to the arguments put forward by McManus and Cason (1950), this colouration of the cytoplasmic granules is due to the presence of 1-2 glycol group, whereas the globules do not possess this group but some other carbohydrate. These globules also give positive test with the ninhydrin-schiff test (Pearse, 1960) indicating the presence of proteins, containing  $\text{NH}_2$  groups. It is possible that these cytoplasmic inclusions of basophil cells are some protein-carbohydrate complex, and further work in this direction is being taken up.

The cells in the pro-adenohypophysis and meta-adenohypophysis are completely PAS-negative. No orange G cell in meso-adenohypophysis, as described by Matty and Matty (1959) in the case of *Pseudoscarus* and *Scarus*, is PAS positive in the present case. Though the neurohypophysis is PAS-negative, the thin connective tissue sheaths enveloping the neurohypophysial branches are moderately coloured, thereby rendering the distributional study of its branches or elements easier. This shows that in *C. mrigala* all the basophils are PAS positive and contain carbohydrates, while no acidophil cell of the adenohypophysis is PAS-positive, i.e., not containing any carbohydrate.

**Proteins**—For the detection of proteins, containing tyrosine, the Millon reaction as modified by Bensley and Gersh (Pearse, 1960) was carried out but this did not reveal the presence of any tyrosine in the pituitary cells. Similar results were obtained when Baker's modification of Millon reaction (Pearse, 1960) was employed. The negative reaction in these two tests suggests that either tyrosine is absent or is not present in sufficient quantity to be detected by these histochemical methods. Ninhydrin-schiff method for protein-bound  $\text{NH}_2$  group (Yasuma and Ichikawa, 1953) carried out on the paraffin sections, however, gave slight positive test with basophilic granules

and moderately positive reaction with the globular bodies of the basophil cells.

Thus it may be inferred that the cytoplasmic inclusions of the basophil cells are carbohydrates containing proteins or glyco-proteins in nature.

*Nucleic acid.*—To detect the presence of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) different techniques as given in the section 'Material and Methods' were employed. The nuclei of red blood cell corpuscles revealed the presence of much DNA in them, whereas no other element of the pituitary cells showed the presence of this acid. Even the nuclei of acidophil or basophil cells gave negative reactions for the acid. The concentration of RNA was also less, because only the basophil granules and the globular bodies were slightly tinged.

*Lipids.*—Sudan black B test in 70% alcohol (Baker, 1949, 1956) or in propylene glycol (Chiffelle and Putt, 1951) was employed on the gelatin sections prepared from the pituitaries of different maturity stages of fish and fixed in formol-calcium with or without postchroming. These gave negative results in all stages except in the V and VI stages (Lal, 1962) where a few round, positive and prominent bodies may be present irregularly. Acid haematin test (Baker, 1949) for phospholipids also showed positive reaction in V and VI stages only where cells along the fine branches of neurohypophysis showed positive granulation. This was negative after pyridine control method. From this it may be concluded that a little quantity of lipids (mostly phospholipids) develop in the later stages only, the presence of which is very obscure and difficult to explain. In the material extracted with hot acetone or ether the positive reaction to Sudan black test is given by the adenohypophyseal cells in all the stages. But in view of the negative results with the material fixed in formol-calcium, these positive results are suspect and cannot be taken into consideration as due to lipids.

#### SEASONAL CHANGES IN THE PITUITARY COMPONENTS

As the development of the gonads proceeds towards the final maturity stage, certain morphological and histological changes take place in the carp's pituitary. The changes observed in *C. mrigala* are described below under three headings, viz., (1) early maturity stage (stages I and II of Lal, 1962); (2) maturing stage (stages III and IV of Lal, 1962); and (3) final maturity stage (stages V and VI of Lal, 1962).

*Early maturity stage.*—The cells of all the regions are small and the neurohypophyseal branches are not very prominent. Blood supply also is

not well developed. The colloidal material is not generally present in the basophil cells of the meso-adenohypophysis. The nerve fibres are very fine.

*Maturing stage*—There is a definite overall increase in the pituitary components. The basophil cells in the meso-adenohypophysis increase in number corresponding with the increase in the size of meso-adenohypophysis. Their size also becomes progressively bigger. Some of these cells show colloid-bodies. Acidophils have decreased in number. The tree-like branches of the neurohypophysis in the meta-adenohypophysis has been established by now. The nerve fibres instead of being finer have become thicker. The suture between meso, and meta-adenohypophysis has made its appearance. The cytoplasm of basophil cells of meso-adenohypophysis becomes more granular and stains intensely. The cells in the pro- and meta-adenohypophysis do not show any marked change. However, the size of these two regions also increases a little.

*Final maturity stage*—The histological changes in the pituitary are very important and prominent when the carp reaches full sexual maturity. The meso-adenohypophysis now occupies twice the area of meta-adenohypophysis. There is a marked increase in the basophil cells of the meso-adenohypophysis and a corresponding decrease in the number of acidophil cells, which are present now in small islets. The basophil cells increase very much in size—the largest measuring twenty micra in diameter. The transitional stages between completely degranulated and fully granulated basophils are available. The basophils in the central region reveal large vacuoles in place of the colloid-bodies, and those at the periphery reveal in each cell one or more colloid-bodies measuring up to twelve micra in diameter. The meta-adenohypophysis does not show any detectable change. However, the blood vessels have increased very much in number and size to cope with the larger size of the region. The coarse fibres of the neurohypophysis are in striking contrast to the fine nerve fibres present in early stages. Degenerative changes in the pituitary basophils like vacuolization, pycnosis, loss of cytoplasmic granules and disappearance of their nuclei are evident.

In the pituitaries collected from fish one hour after spawning the basophil cells at the periphery of the meso-adenohypophysis showed the maximum number of colloid bodies in addition to the above-mentioned degenerative features.

## DISCUSSION

The teleost pituitary exhibits wide variations from species to species with regard to the topographical arrangement of the main lobes of the gland,



the shape and size of the pituitary and its attachment with the brain, the distribution of the nervous elements and blood vessels, and the characteristic cell types present in each region. The attachment of the pituitary with the brain, by means of an infundibular stalk or without it is significant from the view-point of evolutionary status of the gland. In *C. mrigala*, the pituitary has been found to be attached with the brain by means of a short and thick infundibular stalk. This condition is considered to be an advanced condition (Bell, 1938). In the primitive types, the gland is closely attached to the brain floor without any definite stalk (Charipper, 1937). Such a condition is found in *Anguilla* and *Mormyrus* (Stendell, 1914), *Gasterosteus aculeatus* (Bock, 1928), *Trichogaster fasciatus* and *Rhynchobdella aculeata* (Singh and Sathyanesan, 1962). This type of pituitary has been named as type-A and the former as type-B by Kerr (1942 a). Later, Bretschneider and Duyvené de Wit (1947) classified these two types as platy-basic and lepto-basic respectively. The latter authors further classified the lepto-basic type into three categories, viz., caudo-basic, dorso-basic and cranio-basic according to the attachment of the infundibular stalk with the pituitary at the posterior, middle and anterior ends respectively. In *C. mrigala*, the infundibulum is attached at the mid-dorsal point thus placing it under dorso-basic type which is very common and has been reported in *Fundulus* (Mathews, 1936), salmon and trout (Robertson and Wexler, 1962 a, b), *Labeo rohita* (Khan, 1962), *Cirrhina reba*, *Mystus seenghala*, and *Barbus stigma* (Sathyanesan, 1958, 1960 a), *Xenentodon cancila* (Singh and Sathyanesan, 1962) and in the six poeciliids, *Platypoecilus variatus*, *Limia tricolor*, *Xiphophorus helleri*, *Mollienisia sphenops*, *Lebistes reticulatus* and *Mollienisia latipinna* (Potts, 1942).

Fishes generally do not have a sella turcica. However, the present observations indicate that in *C. mrigala*, a well-developed sella turcica is present. Other instances of such a condition are in *Polypterus* (deBeer, 1926), *Epiplatodus* (Griffiths, 1938), and in *Hilsa ilisha* (Misra and Sathyanesan, 1958). Probably this feature is an advanced one and a near approach to higher vertebrate-pituitary development. The large size and the globular shape of the gland may account for the presence of a sella turcica to accommodate the former which naturally, as compared to their linear counterparts, would require more space between brain and the cranial floor.

The orientation of the glandular lobes of pituitary is variable. In the present fish, the pro- and meso-adenohypophysis are present one behind the other along the antero-posterior axis, whereas the meta-adenohypophysis is situated ventral to the former. This type of arrangement of glandular

regions has also been reported in Pacific salmon and rainbow trout (Robertson and Wexler, 1962 *a, b*), which can neither be described as linear, as found in *Trichogaster fasciatus* and *Rhynchobdella aculeata* (Singh and Sathyanesan, 1962) and *Heteropneustes fossilis* (Sundararaj, 1959) nor vertical as in *C. carpio* (Stensell, 1914, Scruggs, 1939), *Carassius auratus* (Bell, 1938, Levenstein, 1939, Scruggs, 1939), *Cirrhina reba* (Sathyanesan, 1958) and in *Labeo rohita* (Khan, 1962). Since the condition of glandular lobes as found in *C. mrigala* has not been designated earlier, the author proposes the term lineo vertical or antero ventral for the same. This probably is an advanced condition over the primitive linear arrangement since it presents a transitional stage between linear and vertical arrangements, the latter being considered to be highly evolved (Bell, 1938).

The pituitary of *C. mrigala* always shows the presence of pro-adenohypophysis. It is recognised that the presence of this lobe and its position with regard to the other lobes present much variations. A few important instances in which such a lobe is absent are *Gadus morrhua* (Herring, 1908, 1913), and *Fundulus* (Mathews, 1936). The anterior position of the lobe in *C. mrigala* is similar to that reported in salmon and trout (Robertson and Wexler, 1962 *a, b*) and in the six poeciliids (Potts, 1942). In *Cirrhina reba* (Sathyanesan, 1958) and in *Labeo rohita* (Khan, 1962), the lobe is dorsal—a position identical to that reported in mammalian pituitaries (deBeer, 1926). The conspicuous empty vesicles described in the pars anterior (pro-adenohypophysis) of *Chanos chanas* (Tampi, 1951, 1963), salmon and trout (Robertson and Wexler, 1962 *a, b*) are totally absent in the present case and this finding is in agreement with the observations of Sathyanesan (1958) in *Cirrhina reba*. Hypophysial cavity is described during the development of *Amia* (Smith, 1914, deBeer, 1923) and *Lepidosteus* (Balfour and Parker, 1882). Kerr (1949) remarks that the presence of a hypophysial cavity in the adult *Acipenser* and in the early stages of *Amia* and *Lepidosteus* shows that its absence in the teleosts is secondary. The closed vesicles of the pars anterior of the primitive teleosts such as eel and trout is found to be the reduced hypophysial cavity (Kerr, 1943). Similar cavities in the pars anterior have been reported by Sathyanesan (1960 *b*) in *Pangasius pangasius*. The absence of a hypophysial cavity in *C. mrigala* may be considered a more advanced condition than that obtained in the primitive teleosts, the eel and trout, where this structure is retained.

The cells in the pro-adenohypophysis of *C. mrigala* are mostly acidophilic with a few occasional dull basophils and chromophobes as reported in *Cyprinus carpio* and *Carassius auratus* (Scruggs, 1951) and in *Labeo rohita*.

(Khan, 1962). Tilney (1911) in eel, Stendell (1914) in *C. carpio* and *Esox lucius*, and Bell (1938) in *Carassius auratus* reported this lobe to be entirely basophilic. However, with the help of more recent techniques, this lobe in the above-mentioned fishes has been shown to be predominantly acidophilic by Florentin and Weis (1931); Scruggs (1939) and Levenstein (1939). Thus the pro-adenohypophysis of teleost fish is greatly different from that of the higher vertebrates in that the former contains mainly acidophils. Ventrally, in the pro-adenohypophysis of *C. mrigala* there are some acidophil cell columns separated by basophils. The latter cells, apparently, appear to belong to pro-adenohypophysis but actually they are the cells belonging to meso-adenohypophysis which probably did not segregate completely during the development of the gland (Scruggs, 1951). Such an inference is possible as these basophil cells contain globular bodies and give similar tests (*vide supra*) with different techniques like those of the peripheral basophils of the meso-adenohypophysis. The cells of this lobe present no special arrangement. The laminae reported by Tilney (1911) in eel and Scruggs (1939) in Salmonidae are absent in the present species. Herring (1908) in *Gadus morrhua* described the arrangement of cells in chords separated by connective tissue. These two conditions are primitive, whereas the former one is an advanced condition (Potts, 1942).

The meso-adenohypophysis is the most important lobe of the pituitary not only in the teleost fish but in all the other vertebrates also and shows much variations with regard to the size and the cell components even in the same fish at different maturity stages. This lobe which grows enormously in size in *C. mrigala* lies posterior to the pro-adenohypophysis with no distinct boundaries or connective tissue septa as shown in *Gasterosteus* (Bock, 1928). The lobe consists mainly of acidophils, some basophils and chromophobes in the early maturity stages. The acidophils are outnumbered by the basophils in the final sexual maturity stages. Such a change in cell components of the lobe is in complete agreement with the observations of earlier authors who have studied the pituitaries of fish in different maturity stages (Scruggs, 1939; Robertson and Wexler, 1962 *a, b*; Sundararaj, 1959; Sathyanesan, 1958, 1960 *a*).

It will be observed from the present account that it has not been possible to differentiate the two types of basophils by adopting the PAS (McManus, 1946) and AF (Gomori, 1950; Halmi, 1952) techniques. This agrees with the findings of Robertson and Wexler (1962 *a, b*). However, it may be pointed out that Atz (1953), Barrington and Matty (1955), Matty and Matty (1959) and Khan (1962) have been able to identify the gonadotrophs from thyro-

trophs by using the same techniques. Thus it is felt that the differentiation of these two types of cells is not always possible by these two methods.

The colloid-bodies in the basophil cells of the meso adenohypophysis of *C. mrigala* show much variation regarding their sizes, number, distribution and the staining properties. Copeland (1943) and Scruggs (1939) have described the acidophilic globules in some of the basophil cells and called these cells as globular basophils, while Sathyanesan (1960 a) called these cells as secretory basophils in case of *Mystus seenghala*, opining that the acidophilic material in these basophil cells, was not present very often, in the form of globules. In the present case the colloid-bodies are always in the form of globules which, however, may be basophilic, acidophilic or amphiphilic (medullary region is acidophilic and cortical region is basophilic). The above terminology, i.e., the globular basophils or secretory basophils, is not satisfactory. The term globular basophils may be taken to mean that the cells are globular in form, whereas basophil cells of various shapes are seen in *C. mrigala* and also in *Labeo rohita* (Khan, 1962). Also, the term secretory basophils may give the impression that these are the only cells engaged in secretory activities, whereas Robertson and Wexler (1962 a, b) have reported that in case of salmon and trout the basophil cells in meso-adenohypophysis are devoid of any colloidal material and still they show secretory activities at different times of maturation and spawning periods. In view of the above arguments, the author proposes the following modified terminology free from any structural and functional complications: (1) acidophilic-colloid basophils, i.e., the basophilic cells having acidophilic colloid-bodies irrespective of their shapes and sizes [as reported by Khan (1962) in *Labeo rohita*, Sathyanesan (1958) in *Cirrhina reba* and Scruggs (1951) in *Fundulus*]; (2) Basophilic-colloid basophils, i.e., the basophilic cells containing basophilic colloidal bodies (as in the present case), (3) amphiphilic-colloid basophils i.e., the basophilic cells possessing the colloid bodies whose medullary regions are acidophilic and the cortices are basophilic (as in the present case), (4) acidophilic-colloid acidophils, i.e., the acidophil cells containing the acidophilic colloid-bodies (as reported in *C. reba* by Sathyanesan, 1958). The colloid bodies in the neurohypophysis and inter-cellular in position may be called acidophilic or basophilic or polychromatic according to the colour reaction. These three types of colloid-bodies have been reported in *C. reba* (Sathyanesan, 1958). The cells without any colloidal material may be termed as acidophils or basophils by virtue of their staining reactions. When vacuoles are present in the cells, they may be called vacuolar acidophils or vacuolar basophils according to their presence in these two types of cells.

The increase in the colloid-bodies of the peripheral basophils of meso-adenohypophysis of a spent fish suggests that the colloidal material in these cells, at least, is not a factor responsible for inducement of spawning in fish. However, the presence of vacuoles in the central basophil cells, which prior to spawning lodged the colloidal bodies, may play some role in the process of breeding. *Cirrhina mrigala* resembles *Carassius auratus* (Scruggs, 1951), *Mystus seenghala* (Sathyanesan, 1960 a) in the occurrence of more basophils with or without colloidal material during the pre-spawning and spawning periods. The net-like or sieve-like appearance of meso-adenohypophysis in the present species during post-spawning period stands in comparison with the shrunken chromophobes of *Fundulus* (Mathews, 1936), net-like appearance in *Xiphias* (Lee, 1942), *Cirrhina reba* (Sathyanesan, 1958), *Mystus seenghala* (Sathyanesan, 1960 a), salmon and trout (Robertson and Wexler, 1962 a, b). Bretschneider and Duyvené de Wit (1947) report the characteristic changes from acidophily of the 'anterior lobe' (meso-adenohypophysis in the present case) to basophily and a return again to acidophily during the pre-spawning phase through a resting phase. These observations are in conformity with the present observations.

The degenerative changes such as vacuolization, pycnosis, loss of cytoplasmic staining of basophil cells of meso-adenohypophysis with disappearance of their nuclei in the spawning *C. mrigala* are in agreement with the observations of the previous authors. Thus, it may be concluded that this lobe, if not exclusively responsible for spawning activities, at least plays a major role towards this end.

The characteristic feature of meta-adenohypophysis is that it is always intimately associated with the neurohypophysis (deBeer, 1926). Such a condition of neurohypophysis has probably reached the same high degree of development in *C. mrigala* as has been reported in *C. reba* and *M. seenghala* (Sathyanesan, 1958, 1960 a) and in *Labeo rohita* (Khan, 1962). The basophils, which have always been reported as being either the sole or main constituents of this region as in *M. seenghala* (Sathyanesan, 1960 a) are conspicuous by their absence in the present species. In this case no basophil or amphiphil cell is present. The only representatives are the acidophils and chromophobes. However, the acidophils can be distinguished into orange G and red-coloured cells as in *Esox* (Scruggs, 1939). Another important feature of this lobe is the presence of blood vessels which, along with the acidophilic nature, may suggest the possibility of the lobe being not meta-adenohypophysis at all. However, the mere fact that this portion of the pituitary is most intimately connected with the neurohypophysial elements, strongly suggests

that it can be nothing else hut the meta adenohypophysis comparable to the pars intermedia of mammals Baumgartner (1915), Tilney (1911) and Stendell (1914) found the cells of intermedia (present meta adenohypophysis) to be acidophilic in Selachii. This lobe in Selachii is highly vascular. In this respect, the meta adenohypophysis of *Cirrhus mrigala* is comparable to that of Selachii. The vascular nature of the lobe has also been reported in skate and dogfish (deBeer, 1926) and *Mystus seenghala* (Sathaynesan, 1960 a). This lobe which contains blood vessels reveals the predominance of acidophil cells. So it is possible that there may be some correlation between the acidophilic nature of this lobe and the blood supply. The comparatively bigger size of the lobe may account for the vascular nature. As the blood vessels and the neurohypophysis are in association in this region, it is likely that the secretions of the neurohypophysis and of this lobe may be carried by blood vessels directly for quick action.

The neurohypophysis in *Cirrhus mrigala* has been found to innervate all the three lobes although the main trunk dividing the meso-adenohypophysis passes down and branches extensively in meta adenohypophysis as reported in *Labeo rohita* (Khan, 1962) and in *C. reba* (Sathaynesan, 1958). In *P. americanus* and *C. carpio* (Scruggs, 1939) the neurohypophysis is exclusively restricted to meta adenohypophysis, whereas in *Fundulus* (Mathews 1936), it is present in the meso- and meta adenohypophysis. The distribution of the neurohypophysis in all the three lobes is considered to be an advanced feature over the restricted supply in meta adenohypophysis (Charipper, 1937). No infundibular cavity has been observed in *C. mrigala*. It may be well developed in some fishes as in *Jenynsia* (Rojas *et al.*, 1934) or it may be reduced as in *R. aculeata* (Singh and Sathaynesan, 1962). Fish possessing no infundibular cavity approach the vertebrate condition.

The basophil cells only give a positive reaction with PAS test revealing the presence of carbohydrates. These cells also show the presence of proteins in accordance with the observations of earlier workers that the basophil cells representing the thyrotrophs and gonadotrophs contain hormones made up of glyco-proteins. Thus the increase in number and size of the basophil cells towards the final maturity stage of the fish and the proportional increase in the secretion of glyco-protein hormones is confirmed.

In conclusion, it may be mentioned that the pituitary gland of *Cirrhus mrigala*, with regard to the morphological and histological nature, reveals some advanced features in the development like the absence of infundibular and hypophysial cavities, the presence of an infundibular stalk, sella turcica,

lineo-vertical arrangement of the glandular regions and the ramifications of the neurohypophysis in all the regions of the adenohypophysis.

#### SUMMARY

1. The fish, *Cirrhina mrigala*, possesses a dorso-basic type of pituitary gland. The structure of the gland is typically piscine, *i.e.*, it consists of neurohypophysis and adenohypophysis. The latter is further differentiated, histologically, into pro-, meso- and meta-adenohypophysis.

2. The histological observations revealed remarkable changes that take place mainly in the meso-adenohypophysis of the gland during different maturity stages. The pituitary in the early maturity stages is characterised by a relatively small meso-adenohypophysis with a predominance of acidophil cells. By the time the gonads enter the final stage of sexual maturity, the meso-adenohypophysis increases much in size and the basophils outnumber the acidophils to the extent that the latter are represented only as small islets. Other areas of the gland show little changes except that the meta-adenohypophysis becomes more vascular.

3. The pituitary of *Cirrhina mrigala* exhibits degenerative changes such as vacuolization and degeneration of the basophil cells.

4. The colloid-bodies of the basophil cells increase in size and number towards the spawning period.

5. Three types of colloid-bodies, *i.e.*, basophils, acidophils and amphiphils have been identified and a modified terminology has been suggested for the cells possessing these colloid-bodies.

6. No distinction between gonadotrophs and thyrotrophs could be made by using PAS and AF techniques.

7. The position of the pituitary lobes has been designated as lineo-vertical—a new terminology suggested to describe similar orientation of pituitary lobes in fish.

8. The possible role of the colloid-bodies in the basophil cells of the meso-adenohypophysis and the highly vascular nature of meta-adenohypophysis has been discussed.

9. To detect the lipids, carbohydrates, proteins and nucleic acid, the histochemical tests carried out and results obtained have been given, which reveal the presence of glyco-proteins in the basophil cells of meso-adenohypophysis of the gland.

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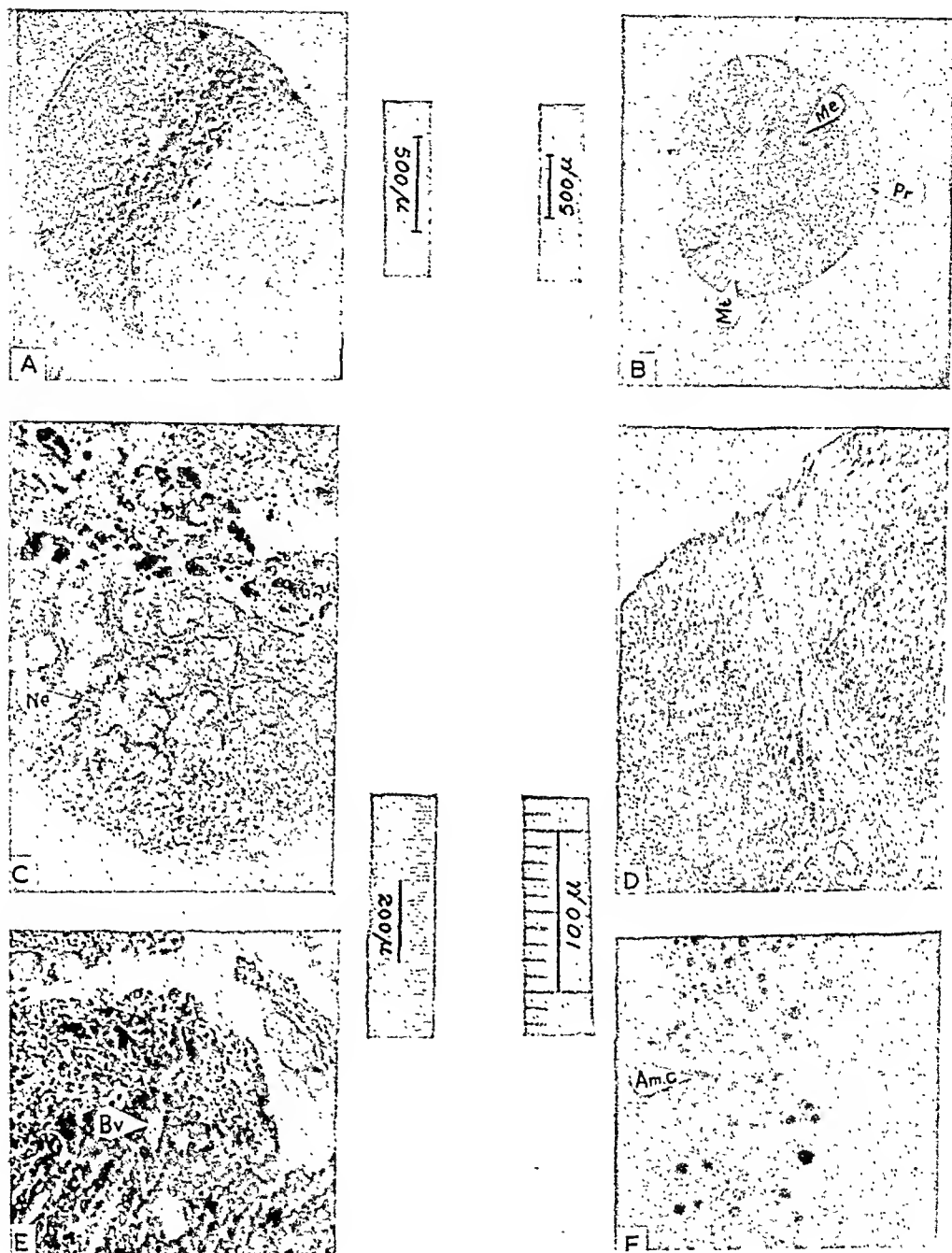
## EXPLANATION OF PLATES

## PLATE XII

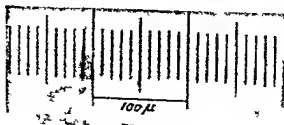
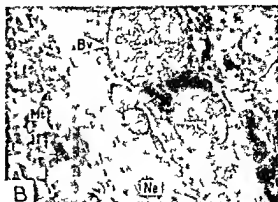
- A. Section of the pituitary gland of *Currhina mrigala* in the early stage of maturity Zenker Periodic acid schiff (McManus, 1946)
- B Median sagittal section of pituitary gland showing four distinct regions or lobes (1) pro-adenohypophysis, (2) meso-adenohypophysis, (3) meta-adenohypophysis, (4) neurohypophysis. Zenker Mallory Heidenhain
- C. Portion of a median sagittal section through the pituitary gland. Note the tree-like branching of the neurohypophysis in the meta-adenohypophysial region. Zenker Halmis modification of aldehyde-fuchsin technique with potassium permanganate oxidation
- D Portion of a median sagittal section through the pituitary gland showing the neurohypophysis, with nerve fibres and blood vessels, passing down through the middle of the meso-adenohypophysis Zenker Mallory Heidenhain.
- E. Section of the gland showing the blood vessels in the neurohypophysis and meta-adenohypophysis. 80% ethanol Mallory's triple
- F Peripheral region of meso-adenohypophysis of the pituitary gland showing the dupol nature of colloid-globules in the basophil cells. Bouin, Foots modification of Masson's trichrome
- Am. C, Amphiphilic colloid bodies, Bv, Blood vessel, Me, Meso-adenohypophysis, Mt, Meta-adenohypophysis, Ne, Neurohypophysis, Pr Pro-adenohypophysis.

## PLATE XIII

- A Section through the ventral portion of pro-adenohypophysis showing acidophilic cell-columns separated by a basophilic cell column, the latter belonging to meso-adenohypophysis. Bouin Mallory's triple
- B Portion of the neurohypophysis showing large blood vessels which contain red blood corpuscles. 80% ethanol, Mallory's triple



FIGS. A-F



FIGS A-F

- C. A portion of the peripheral region of meso-adenohypophysis. Note the colloid-bodies directed towards a branch of neurohypophysis. Bouin; Periodic acid-schiff. .
- D. Central region of meso-adenohypophysis of the pituitary gland from a fish in an advanced maturity stage. Note that some cells are vacuolated while the others contain the colloid-bodies. Bouin: Mallory's triple.
- E. Peripheral region of meso-adenohypophysis of the pituitary gland from a spent fish, showing the increase in number and size of the colloid-bodies in the basophil cells. Compare with Plate II C. Bouin: Periodic acid-schiff.
- F. Central region of meso-adenohypophysis from a fish in the final stage of sexual maturity. Note the vacuolated basophil cells, blood vessels, blood sinusoids, and general degeneration of cells. Bouin: Heidenhain iron haematoxylin.

*Ac.*, Acidophilic cell; *Bc.*, Basophil cell; *Bv.*, Blood vessel; *Bs.*, Blood sinusoid; *Cb. B.*, Colloid-bodies in basophil cell; *Me.*, meso-adenohypophysis; *Mt.*, meta-adenohypophyiss; *Ne.*, Neurohypophysis.

# A STUDY OF ALLOXAN-INDUCED "DIABETES" IN THE ESTUARINE CLAM, *MERETRIX CASTA* (CHEMNITZ)

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(Communicated by Prof R. V Seshaiya, F A.Sc)

## INTRODUCTION

In a previous paper, the author (1963) described that injection of insulin into the clam, *Meretrix casta* (Chemnitz) produces effects similar to what happens in vertebrates and contrary to the view generally held in regard to the effects of insulin in molluscs. As a continuation of this study in a programme bearing on the regulation of carbohydrate metabolism in clams, the author undertook a study of the effect of alloxan injection in these animals.

In investigations relating to diabetes and regulation of carbohydrate metabolism in vertebrates, the alloxan technique has made a significant contribution. Alloxan, which is structurally related to pyrimidine and is the ureide of mesoxalic acid, was first shown in 1937 to produce hyperglycaemia and death in rabbits. It was later shown that alloxan has a specific destructive effect on beta cells of the islet tissue of pancreas and thereby produces experimental diabetes. This is in contrast to the effect of the diabetogenic hormone from the pituitary or partial pancreatectomy which is said to result in hyperactivity of the beta cells. All these studies have been entirely limited to vertebrates. It was thought, therefore, that alloxan experiments on an invertebrate like the clam, *Meretrix casta*, might be interesting. Alloxan-diabetes in relation to liver glycogen has been studied in albino rats and the effects of glucose and insulin on the diabetogenic action of alloxan on dogs have been studied by Arteta *et al* (1954).

The present account describes the effect of alloxan injection in *Meretrix casta*.

## MATERIAL AND METHODS

Specimens of *Meretrix casta* were collected from the Vellar estuary. The weight of the specimens used in these experiments varied from 3 gm

to 8 gm. each. Crystalline alloxan of analytical purity (kindly donated by Dr. A. E. Mirsky of Rockefeller) was used to produce experimental diabetes. The specimens were starved for 24 hours prior to experimental treatment as starvation is known to confer on animals greater susceptibility to alloxan effect.

In the initial experiments, the alloxan dose injected into the specimens was 1 mg./5 gm. body weight. In subsequent sets of experiments, higher doses varying from 2 mg./5 gm. weight, 3 mg./5 gm. weight and 4 mg./5 gm. weight were used. Alloxan was injected into the foot as quickly as possible. The time required for alloxan effect was determined by trial and by estimating the blood glucose. The normal level of blood glucose in *Meretrix casta* is  $47.9 \pm 2.52$  mg./100 ml. of blood, and when the glucose content rose to 70 mg./100 ml. of blood following the injection of alloxan, it was assumed that the diabetogenic effect had resulted.

The glucose content of the blood and the glycogen content of the foot and digestive diverticula were estimated at intervals. Following an injection of alloxan 1 mg./5 gm. weight, the glucose and glycogen were estimated at four successive hourly intervals. With higher doses, the estimations were done after 2 hours.

## METHODS

Glycogen was estimated adopting the procedure described by Kemp and Kits (1954) and as described in a previous paper by the present author (1963).

Controls were maintained in all these experiments. In these distilled water was injected, equal in volume to that of alloxan injected.

Besides the experiments with alloxan injection mentioned above, the effect of a toxin, T.A.B. vaccine (*Salmonella typhi*) and also the effect of low temperature like 4° C. on the glycogen content of alloxan diabetic animals was determined.

Experiments were also conducted to determine the minimum time required for the inhibition of diabetes when alloxan injection was preceded by glucose injection into the animal.

## RESULTS AND OBSERVATIONS

The normal glycogen content of the digestive diverticula and foot is  $1.2855 \pm 0.068$ ,  $0.4362 \pm 0.022$  respectively. When 1 mg./5 gm. wt, of

TABLE I

*Effect of injection of 1 mg/5 gm wt of alloxan on tissue glycogen and blood glucose of Meretrix casta after 24 hours of Starvation*

State of the animal	Glycogen in mg /100 mg in Foot				Glycogen in mg /100 mg in digest ve diverticula				Blood glucose mg /100 ml			
	1st hr	2nd hr	3rd hr	4th hr	1st hr	2nd hr	3rd hr	4th hr	1st hr	2nd hr	3rd hr	4th hr
Normal	0.436 ± 0.022	0.436 ± 0.022	0.436 ± 0.022	0.436 ± 0.022	1.286 ± 0.068	1.286 ± 0.068	1.286 ± 0.068	1.286 ± 0.068	47.9 ± 2.5	47.0 ± 2.5	47.0 ± 2.5	47.9 ± 2.5
Alloxan diabetic	0.870 ± 0.119	0.883 ± 0.071	0.551 ± 0.032	1.111 ± 0.963	1.376 ± 0.067	1.836 ± 0.162	1.740 ± 0.132	3.037 ± 0.143	100 ± 23	81 ± 9	92 ± 21	43 ± 1
Control	0.494 ± 0.025	0.494 ± 0.032	0.510 ± 0.128	0.471 ± 0.056	1.290 ± 0.134	1.302 ± 0.093	1.249 ± 0.171	1.212 ± 0.090	46.8 ± 14	46.0 ± 4	47.6 ± 2	51.2 ± 2

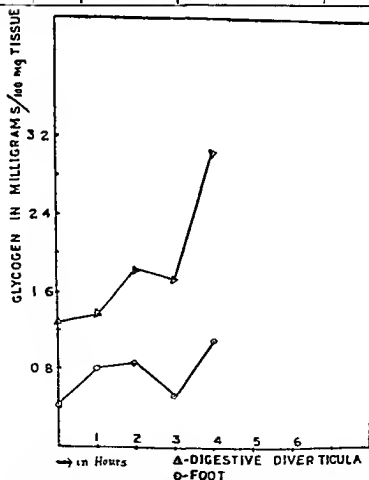


FIG 1 Effect of injection of 1 mg/5 gm. wt. alloxan, on glycogen content in tissues



alloxan is injected, the overall effect is an increase of glycogen both in the foot and digestive diverticula (Table I, Fig. 1). However, there are certain striking differences between what happens in the foot and digestive diverticula. During the first hour, the foot shows a more pronounced increase of glycogen content than the digestive diverticula in which the rise is only slight. In the following hour a pronounced increase is seen in the digestive diverticula, whereas in the foot it is slight. In the third hour, there is a fall of glycogen content in both the tissues but is greater in the foot. In the fourth hour there is a steep rise of glycogen in digestive diverticula but less in the foot. Now the increase in glycogen in both foot and digestive diverticula is higher than in the previous hours.

The injection of increased doses of alloxan also shows some interesting results (Table II, Fig. 2). When 2 mg./5 gm. wt. are injected, the increase

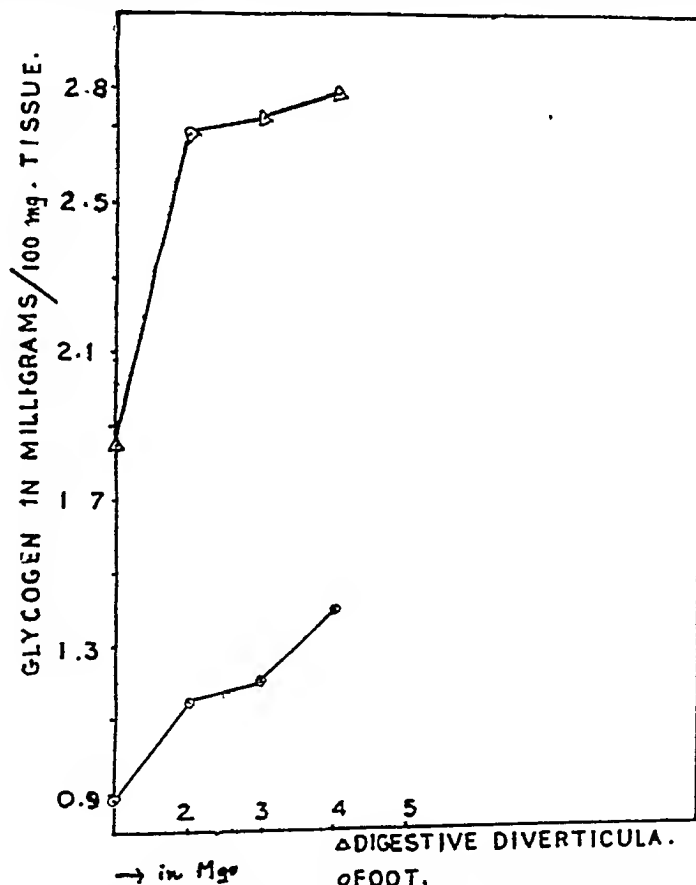


FIG. 2. Effect after 2 hours injection of different doses of alloxan on glycogen content tissues.

TABLE II  
*Effect after 2 hours of injection of different doses of alloxan on glycogen content in tissues of 24 hours starved Meretrix casta (Chemnitz)*

Tissue	Alloxan diabetic				Control			Normal
	2 mg	3 mg	4 mg	4 mg	2 mg	3 mg	4 mg	
Foot (Glycogen in mg / 100 mg wt)	1 153± 0 052	1 202± 0 082	1 393± 0 104	0 914± 0 079	0 988± 0 126	0 973± 0 099	0 836± 0 016	
Digestive diverticula (Glycogen in mg /100 mg wt)	2 688± 0 128	2 723± 0 171	2 778± 0 123	2 063± 0 216	1 914± 0 137	1 936± 0 124	1 827± 0 029	

in the glycogen after two hours is strikingly greater than the action on 1 mg./5 gm. wt. of alloxan in one hour. It is also observed that the increase is greater in the digestive diverticula than in the foot. When 3 mg./5 gm. wt. is injected, there is only slight increase over what is seen when 2 mg./5 gm. wt. is injected. But the larger doses result in slightly greater increase of glycogen in foot than in digestive diverticula.

*Effect of toxin, T.A.B. vaccine (Salmonella typhi).*—This toxin was injected in two doses of 0.1 ml. each at intervals of 15 minutes into clams, which were starved and treated with alloxan to produce experimental diabetes. The injection of toxin resulted in glycogen deficiency. There was not only no increase of glycogen, but a definite decrease of glycogen below a normal level was observed.

Cold treatment of alloxan diabetic clams, *i.e.*, of subjecting them to 4° C. for four hours, also produces a diminution of glycogen content below the normal level. The results are shown in Table III. The changes produced in the foot are slight as compared to those in the digestive diverticula. These experiments indicate that the alloxan effect differs from toxic and cold effects.

TABLE III

*Effect of toxin and cold treatment on blood glucose and glycogen content of alloxan-diabetic Meretrix casta (Chemnitz)*

Tissue	Alloxan diabetic			Control			Normal		
	Toxin	Exposure to cold for 4 hours	Recovery	Toxin	Exposure to cold for 4 hours	Recovery	Toxin	Exposure to cold for 4 hours	Recovery
Foot (Glycogen in mg./100 mg. wt.)	1.201 ± 0.269	1.123 ± 0.042	1.174 ± 0.074	0.990 ± 0.010	1.211 ± 0.029	1.112 ± 0.119	1.271 ± 0.053	1.152 ± 0.061	1.152 ± 0.061
Digestive diverticula (Glycogen in mg./100 mg. wt.)	2.093 ± 0.620	2.102 ± 0.087	2.169 ± 0.070	1.883 ± 0.305	2.113 ± 0.069	2.199 ± 0.277	2.846 ± 0.056	2.115 ± 0.034	2.115 ± 0.034
Blood (Glucose mg./100 ml.)	32 ± 2.4	105 ± 1.5	..	47 ± 14	68 ± 8.4	..	48 ± 2.5	48 ± 2.5	..

*Effect of injection of insulin into the diabetic clam.*—When insulin is injected in alloxan-induced diabetic clam, the glycogen content in foot and digestive diverticula shows a greater increase than in only alloxan treated animals. There is also a concomittant decrease in blood glucose level in these animals as is shown in Table IV.

TABLE IV

*Effect of injection of insulin in alloxan diabetic Meretrix casta (Chennai)*

Tissue	24 hours fast without insulin		24 hours fast with insulin	
	Alloxan diabetic	Control	Alloxan diabetic	Control
Foot (Glycogen in mg/100 mg wt)	0 883±0 030	0 796±0 025	1 218±0 116	0 923±0 087
Digestive diverticula (Glycogen in mg/100 mg wt)	1 836±0 172	1 798±0 126	2 722±0 130	2 012±0 237
Blood (Glucose mg/100 ml)	100±23	46 8±14	50±7 9	48 6±6 5

TABLE V

*The action of glucose upon the effects of diabetogenic doses of alloxan in Meretrix casta*

Experimental group	Interval between glucose and alloxan injection	Alloxan dose	No and sex of treated clams		No and sex of developing diabetes	
			Male	Female	Male	Female
1 Previous injection of 5 mg/5 gm wt	5 Minutes	1 mg/5 gm wt	5	5		
2 Previous injection of (a) 5 mg/5 gm wt	15 Seconds	1 mg/5 gm wt	3	2	.	.
(b) 5 mg/5 gm wt	30 Seconds	Do	3	3	..	..
3 Injection of alloxan dissolved in 10 ml of 10% glucose		Do	5	5	.	.

*The action of glucose upon the effects of diabetogenic doses of alloxan.*—The injection of 5 mg./5 gm. wt. of glucose 5 minutes before alloxan injection inhibited the production of diabetes. A number of experiments were carried out in order to determine the minimum time required for this glucose inhibition of alloxan effect (Table V). The results showed that clams which received a total of 5–10 mg. of glucose 15–30 seconds before the injection of 1 mg./5 gm. wt. of alloxan were completely protected against the diabetogenic action of the latter.

Injection of alloxan in glucose medium into *Meretrix* does not produce diabetes. Arteta *et al.* (1954) have reported that such injections into dogs produce diabetes in males only but not in females.

#### DISCUSSION

The observations recorded above bring out some interesting features of alloxan effect on *Meretrix*.

1. Alloxan injection increases the level of blood sugar. In other words it produces diabetes as it does in vertebrates.

2. As a result of alloxan injection the glycogen content increases in the tissues. A similar effect has been observed by investigators on vertebrates.

3. Alloxan has a differential effect on the glycogen content of foot and digestive diverticula. The response of the foot and digestive diverticula to the alloxan injection is not in the same degree.

4. It would appear as though the two tissues had differential limits to glycogen potential. Glycogen reaches a higher level in the digestive diverticula than in the foot as a result of alloxan injection. The overall range of fluctuation of glycogen content in the digestive diverticula is greater than in the foot.

5. Alloxan effect is not due to a toxicity.

6. The puzzling feature is that while blood glucose is decreased in insulin injection, but increased in alloxan injection, the glycogen content of the tissues is increased both in insulin injection as well as in alloxan injection.

We do not have as yet a satisfactory explanation for the increased glycogen synthesis in insulin injections. Krahle (1961) proposed that insulin acts to bring about alteration of the fine structure of responsive cells, resulting in a decompartment which favours glycogen synthesis, fat synthesis and protein synthesis. As he himself remarked this speculation can neither be

proved nor disproved "Methods with the potential for localization of individual cellular and hormone molecules will ultimately be required. The mechanism of hormone action has thus become a problem in solid state-physics" (Krahl, 1961)

Weber (1946), Tuerkishcher and Wertheimer (1947) and others, who investigated the alloxan effect in vertebrates, have remarked that the increase in glycogen in alloxan diabetic animal is due to utilisation of precursors for additional synthesis of glycogen. What these precursors precisely are has not been determined. Apart from this, there is an interesting aspect which has to be investigated in *Meretrix*. Alloxan destroys beta cells in vertebrate pancreas. But on what does it act in *Meretrix*? This is under investigation.

### SUMMARY

1 Effects of different concentrations of alloxan on the blood glucose and tissue glycogen levels of the estuarine clam, *Meretrix casta* (Chemnitz), have been investigated.

2 In alloxan diabetic specimens, there is an increase in the glycogen content in the foot and digestive diverticula. The overall increase and the range of fluctuations was greater in the digestive diverticula.

3 Alloxan shows a differential effect on the quantity of glycogen in the tissues.

4 Alloxan effect on *Meretrix casta* is different from toxic and cold effects on the glycogen content.

### ACKNOWLEDGEMENT

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\* Not seen in original.

# CONTRIBUTION TO OUR KNOWLEDGE OF THE SPOROPHYTE OF *TECTARIA AMPLIFOLIA* (V.A V.R.) CHRISTENSEN

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## INTRODUCTION

THE genus *Tectaria* Cav according to Copeland (1947) includes " 212 species common in all moist tropical lands and remarkably restricted to them, ranging north to the Himalayas, Formosa and Florida, South to Queensland and the Transvaal " Nearly 150 species extend from India to Polynesia according to him The exact number of species present in India is not definitely known, although *T. cicutaria* (L.) Cop., *T. polymorpha* and *T. simon-stium* are commonly cultivated in Indian gardens The species *T. amplifolia* was available in the living state in the departmental garden and was therefore chosen for detailed study

## MATERIAL AND METHODS

Material studied was obtained from the potted plants and was preserved in Formal-acetic Alcohol Sections were cut at 10 to 15  $\mu$  thickness Stains used were Safranin, fast green, and Haematoxylin Maceration in a solution of Nitric acid and Potassium chlorate was done to clear the tissues For the study of venation the leaves were cleared in Methyl Salicylate after dehydration

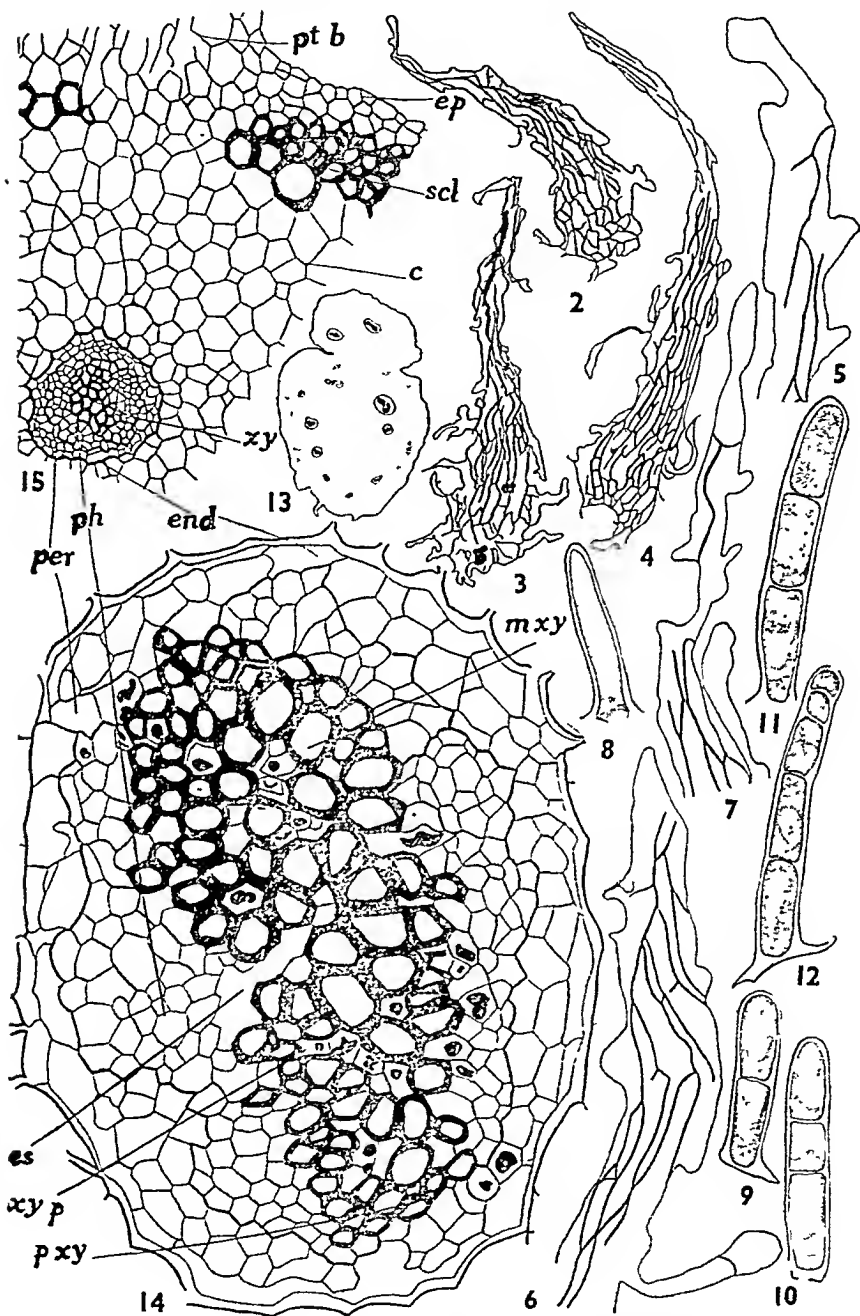
For spore morphology slides were prepared by the acetolysis method of Erdtman (1952) Average spore measurements were taken from twenty readings in each plane, excluding the thickness of the perine

## GENERAL MORPHOLOGY

The plant (Photo 1) is about two feet high with an erect rhizome, and leaves which are pinnately compound The rhizome is short densely covered by adventitious roots which appear all round, densely paleate, also covered

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Figs. 1-15. Figs. 2, 3 and 4. Dermal appendages,  $\times 20$ . Figs. 5, 6, 7. Tips of the dermal appendages,  $\times 104$ . Figs. 8-12. Stages in the development of dermal hair,  $\times 104$ . Fig. 13. A topographic diagram of the T.S. of rhizome (under dissecting microscope). Fig. 14. A mature vascular bundle of the rhizome,  $\times 104$ . Fig. 15. A portion of the T.S. of rhizome,  $\times 26$ .

(c = cortex, end = endodermis, ep = epidermis, es = empty spaces, m.xy = metaxylem, per = pericycle, ph = phloem, p.xy = protoxylem, scl = sclerenchymatous cortex, xy = xylem, xy.p = xylem parenchyma.)

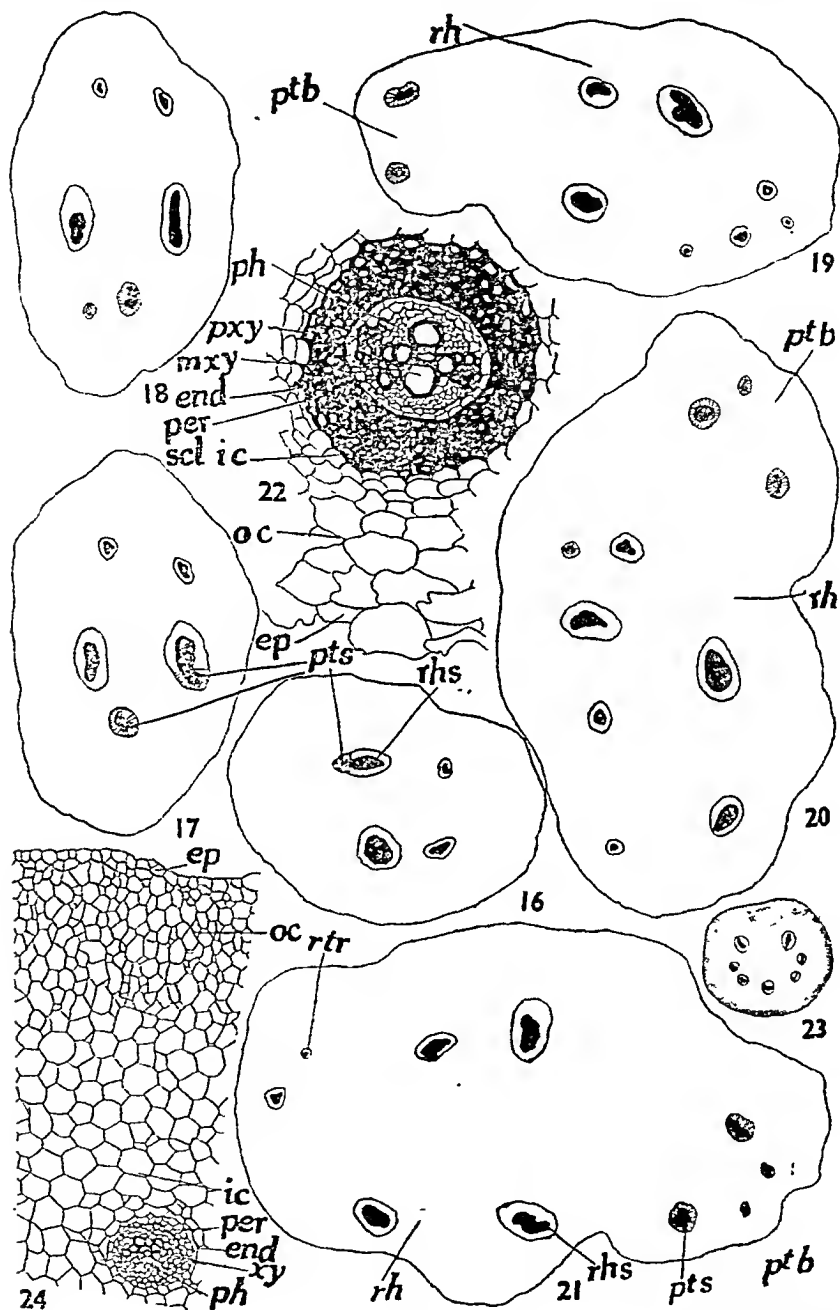
by uniseriate hairs. Dermal appendages are articulate, scale-like and brown in colour (Figs 2-4). They are one cell thick, narrow, broad at the base and tapering towards the end, the margins being uneven and distantly hairy. The cells are irregular but slightly elongated in form. The tip of the scale (Figs 5-7) is only one cell thick and the end cell may be irregular in form, and may be even lobed. The marginal hairs are few, distant, irregular in outline, unicellular and often with bulbous tips. In some of the scales (Figs 3, 4 and 7) the central cells are often slightly thick walled and a black deposit like phlobaphene is often found in the tip region. Apart from these scales and paleae, are also found uniseriate hairs (Figs 8-12) occurring densely all over the plant parts. These cells are generally colourless and some are in a state of division. They have a flat basal attachment and the apical cell is generally round. Uniseriate hairs are also present on the midrib and young parts of the plant as in the allied genus *Cystopteris* (Palser and Benck, 1941).

### RHIZOME

The short, erect rhizome is clothed with scale-like articulate appendages. The rhizome is polystelic in the adult stages (Fig 13 and Photo 2). Usually with 3 to 7 or even more vascular bundles. In each bundle (Fig 14 and Photo 3) the xylem mass is generally exarch diarch. The phloem is mixed with parenchyma and is surrounded by an indistinct pericycle layer. The endodermis is distinct (Fig 15) with casparian strips on the radial walls of the cells, and the outer tangential walls are slightly thickened. The ground tissue consists of parenchymatous, isodiametric cells, without any intercellular spaces. Near the periphery is a band of sclerenchyma, which is interrupted opposite each petiole base. The transverse section shows in cortical cells abundance of starch, which obviously is the principal food reserve. The metaxylem tracheids are mostly scalariformly thickened.

The rhizome in transverse section as already stated shows 6-8 meristeleas as in the allied genus *Peranema* (Davie, 1912). Of these, some are main bundles and the rest are the lateral bundles cut off from the main bundles. Some of these bundles may also be flattened. The same features have been observed in *Diacalpe* and *Peranema* also (Davie, *loc. cit.*)

The main bundles are somewhat larger and placed more or less laterally while the lateral bundles are mostly present on the ventral side of the rhizome between the main bundles (Fig 13).



FIGS. 16-24. Fig. 16-21. Serial transverse sections of the rhizome showing the departure of the petiole trace (all magnified 8 times). Fig. 22. A portion of the T.S. of root,  $\times 26$ . Fig. 23. Topographic T.S. of petiole (under dissecting microscope). Fig. 24. A sector of T.S. of petiole near the base,  $\times 26$ .

(end = endodermis, ep = epidermis, ic = inner cortex, m.xy = metaxylem, o.c. = outer cortex, per = pericycle, ph = phloem, pt.b. = petiolar base, pt.s. = petiolar stele, p.xy. = protoxylem, rh. = rhizome, rh.s. = rhizome stele, r.tr. = root trace, scl. = sclerenchymatous cortex, xy = xylem.)

The main bundle is exarch, protostelic (Fig 14) and slightly plate like, often divided into two groups by an empty space (*es*) formed by the disintegrated or torn parenchyma. The protoxylem points are two and occupy the opposite ends of the xylem plate. The metaxylem has scalariform thickening, and some of the metaxylem tracheids contain a black deposit (Fig 14). Phloem surrounds the entire xylem except at the protoxylem points, in a thin layer. Parenchyma occurs in the phloem as well as xylem. The pericycle and the endodermis are very clear. The latter is made up of thin walled, elongated narrow cells with clear casparian strips in the radial walls. The outer tangential wall is generally thickened.

Serial transverse sections (Figs 16-21) show that the petiolar trace is derived from the main bundles of the rhizome and not from the lateral bundles. Each main bundle cuts off a small petiolar trace (Fig. 16-17, *pts*). These move outwards and pass into the petiolar base (Figs 18 and 19, *ptb*). The bundles, as they ascend the petiole, divide repeatedly (Figs 20 and 21), so that at higher levels the petiole shows several vascular bundles in a transverse section (Fig 23).

### Root

The roots are thin, dark brown and profusely branched. They arise from the rhizome all round the petiolar base as in *Peranema* (Davie, 1912). In the transverse section of the rhizome the root traces (*rt*) can be recognised (Fig 21) near the periphery. A transverse section of the root (Fig 22) shows a thin walled outer cortex enclosing a thick walled inner cortex. The endodermis is single layered and the stele is diarch with four to eight metaxylem tracheids and two protoxylem patches, spreading out. The phloem shows a little phloem parenchyma. No root hairs are seen.

### LEAF

The leaf is pinnately compound with variable number of pinnae, but generally four to five pairs (Fig. 25), often the much lobed lowest pair missing. The pinnae have a crenulate margin, and are hairy on the lower surface.

The petiole is nearly one foot long, cylindrical, covered by hairs all over and also scales at the base. A transverse section of the petiole at the base (Fig 23) shows three main vascular bundles derived from the stele of the rhizome as mentioned earlier. Besides, it also shows (Fig 24) an outer zone of small thick walled, compactly arranged cells and an inner zone of large, thin walled closely packed cells. The outer zone further becomes thick walled

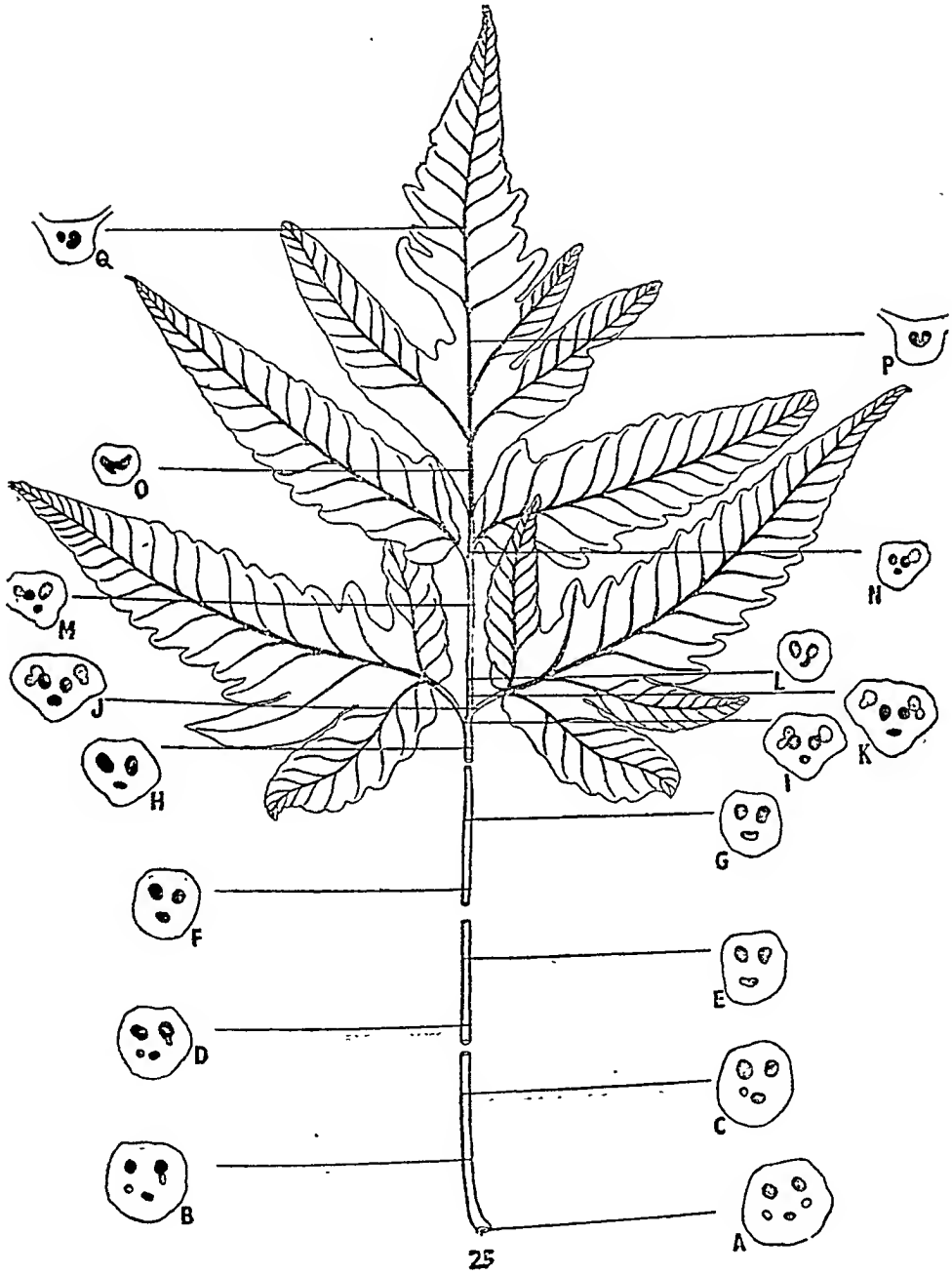


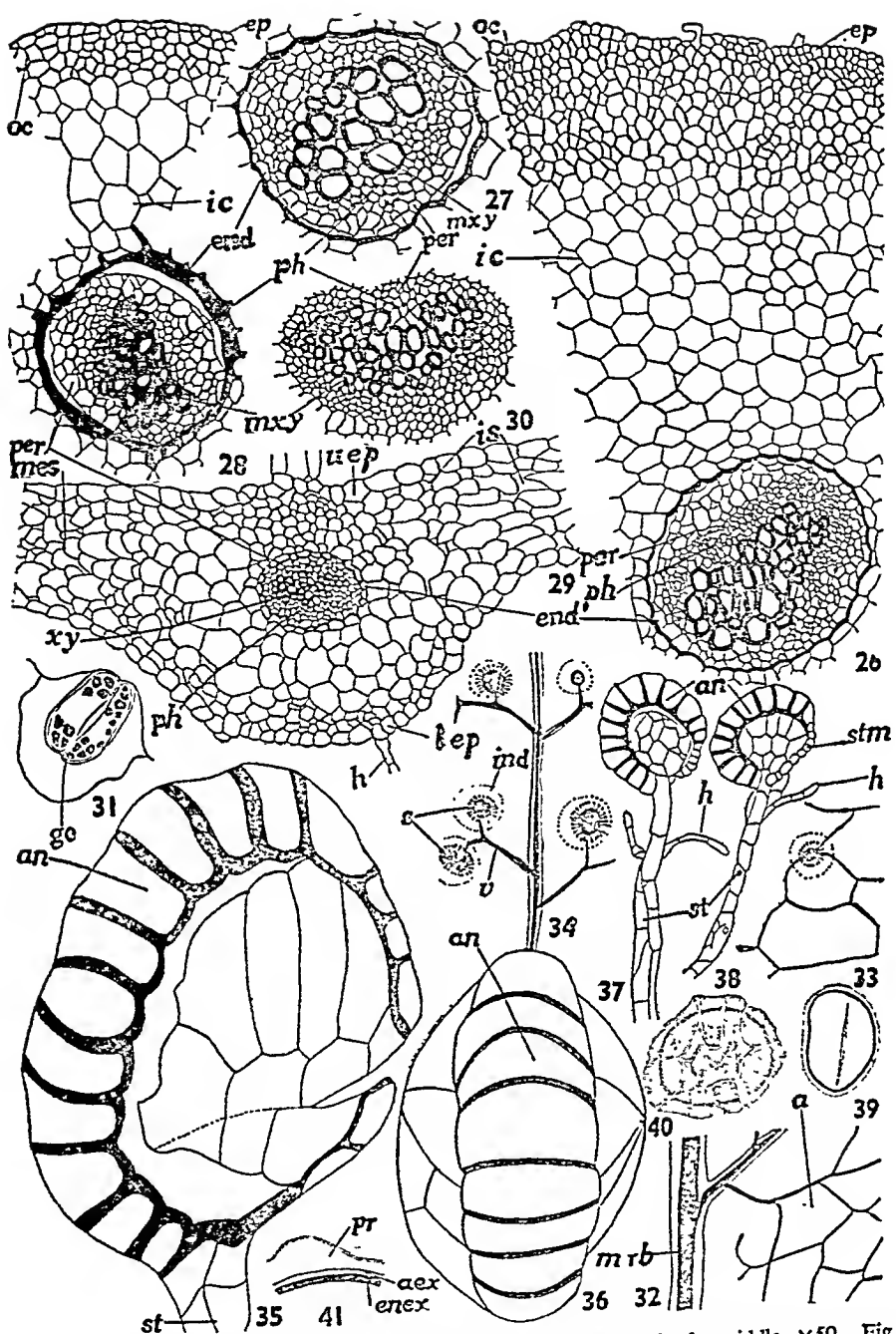
FIG. 25. Diagrammatic representation of the leaf with camera lucida sketches with the help of the dissecting microscope, of the Sections. A-Q taken at different levels.

higher up the petiole. It is probably this lignification that imparts a brown colour to the upper regions of the rachis.

Serial sections of the petiole taken from the base show a definite pattern in the origin of pinna trace. Figure 25 represents a diagrammatic sketch of a leaf with camera lucida sketches of sections taken at the various levels indicated therein. At the very base the transverse section of the petiole (A) shows five bundles, three being main (dark) and two lateral (stippled) ones, as in the allied genera *Peranema* and *Diacalpe* (Davie *loc. cit.*). At a little higher level (B right) one of the lateral bundles comes in contact with one of the main bundles. At a still higher level this actually merges with the main bundle and its identity is lost completely (C). Higher up the lateral bundle on the left comes nearer the central main bundle (D) and fuses with it (E). The nature and significance of these lateral bundles fusing with the main ones could not be understood. Sections taken higher up (Figs F, G and H) show only three main bundles. A little below the first pinna the lateral main bundles abstrict off smaller bundles (stippled). The one on the left-hand side (I) splits into two, one supplying the main part of the pinna on the left and the other, the smaller basal pinnule. Still higher up (J) the bundle abstricted from the main lateral bundle has split and one goes into the pinna proper and the other into the basal pinnule on the right side. Serial sections between the first and second pair of pinnae show the same feature as noted in the basal part of the petiole. K represents a section with three main bundles and two lateral ones (stippled) derived from two of them. At a higher level L these two derived bundles disappear and one of the median bundles unites with one of its fellow-bundles and separates again at a slightly higher level (M). Lateral bundles (stippled) arise on the left and right and supply the second pair of pinnae (M and N). Still higher up (O) the main bundles unite into a common 'V'-shaped bundle (P) which may split up into two or three bundles one of which may go to supply the other pinnules (Q) if any.

The above facts were verified and what stands out clearly is that the earlier lateral bundles derived from the separated main bundles split up into two or more according to the size and lobing of the pinnae. Higher up the pinna traces are simple and are derived from a common 'V'-shaped vascular bundle, formed by the union of the three bundles, seen at the base. But what cannot be explained is the fact that bundle separated from the main bundles fuse again with it.

A transverse section in the middle of the petiole (Fig. 26 and Photo 4) shows the same tissues as above but with further sclerization of the outer cortex as well as the thickening of the outer wall of the endodermis. The



Figs. 26-41. Fig. 26. A portion of the T.S. of petiole in the middle,  $\times 50$ . Fig. 27. Vascular bundle of the petiole,  $\times 50$ . Fig. 28. A portion of the T.S. of petiole at the tip,  $\times 26$ . Fig. 29. T.S. of the pinna,  $\times 26$ . Fig. 30. Stele of the pinna,  $\times 50$ . Fig. 31. Siccata with subsidiary cells,  $\times 104$ . Fig. 32. Venation of the leaf (under dissecting microscope). Figs. 33 and 34. Arrangement of sorus (under dissecting microscope). Fig. 35. Mature sporangium, sur-face view,  $\times 104$ . Fig. 36. Mature sporangium, lateral view,  $\times 104$ . Fig. 37. Sporangium bearing two hairs on the stalk,  $\times 26$ . Fig. 38. Sporangium bearing one hair on the stalk,  $\times 26$ . Fig. 39. Equatorial view of the spore,  $\times 100$ . Fig. 40. Polar view of the spore,  $\times 100$ . Fig. 41. Exine structure and perine,  $\times 340$ .

(a = areole, a.ex = ectoexine, an = annules, en.ex = endocline, end = endodermis, ep = epidermis, g.c. = guard cell, h = hair, i.c. = inner cortex, ind. = indusium, i.s. = inter-cellular space, lep. = lower epidermis, mes. = mesophyll, mxy = metaxylem, mrb = midrib, o.c. = outer cortex, per = pericycle, ph = phloem, pxy = protoxylem, pr = perine, s = sorus, st = stalk, stm = stomium, u.ep. = upper epidermis, v = vein.)

vascular bundle is monarch densely packed with scalariform tracheids. The phloem surrounds the xylem except over the metaxylem end. In some bundles (Fig. 27) the xylem may be loosely packed and the protoxylem position is not easily determined. Generally the vascular bundles are all exarch with scalariform elements in the metaxylem.

A transverse section of the terminal pinna shows that there are uniseriate hairs above and below the midrib (Fig. 29). The mesophyll is undifferentiated. The stomata are confined only to the lower side of the lamina. The mesophyll cells are compact above the vascular bundle, are irregular in form and loosely arranged all over with intercellular spaces. The vascular bundle has a clear endodermis and indistinct pericycle. The xylem is in the form of a plate (Figs. 29-30) often loosely arranged, the protoxylem being generally exarch.

The epidermal cells have thin walls and each stoma (Fig. 31) is surrounded by one or two subsidiary cells. The guard cells are longish and the pore is narrow and elongated.

Venation is essentially pinnate but from the secondary veins branchlets arise forming meshes of areoles. The areoles often having a single or dichotomised, included veinlet, with slightly dilated tip (Fig. 32).

### SORUS

The sorus in *T. amplifolia* is abaxial, superficial and indusiate, the indusium being umbrella shaped, hairy and terminating in small branches of the veins (Fig. 34) or sometimes situated above a point where the veins unite (Fig. 33). The ripe sporangium (Figs. 35-36) is pear-shaped and has vertical incomplete annulus with about 14 to 18 cells, measuring  $165\mu$  to  $234\mu$  in breadth. The stalk is made up of two rows of cells. A third row of the stalk extends only up to a part of the distance from the top. The slit is oblique. Sometimes one or two short hairs are seen on the sporangial stalk (Figs. 37-38 and Photos 5 and 6) as in *Diacalpe* (Davie, 1912). Cells of the sporangial stalk vary in shape and size.

The ripe spores (Figs. 39 and 40 and Photos 7 and 8) are light brown in colour, homosporous, bilateral, slightly planoconvex (P), ovate (e) and measure  $33 \times 42 \times 30\mu$  (E.) in size (range  $24 \times 36 \times 18\mu$  to  $42 \times 54 \times 36\mu$ ). In equatorial view (Fig. 40) the spore is slightly planoconvex with thin transparent perine thrown into irregular folds. The perine-fold is  $4.9\mu$  broad. Exine shows a thick smooth endo-exine (Fig. 41) and an equally broad ecto-



exine. The spores are monolete, the leasure is long and narrow being  $16\mu$  long and  $1.4\mu$  broad. The ripe acetolysed spore is light brown with a slightly darker exine. The acetolysed spores are bigger in size than the unacetolysed ones. The perine is light brown, faintly granulate and thrown into irregular folds.

## DISCUSSION

The genus *Tectaria* Cav. has been differently treated for its systematic position by different workers.

Bower (1926) on the basis of soral characters places it under Superficiales. Holttum (1947-49) follows Christensen (1933) and establishes a distinct subfamily Tectarioideae under the family Dennstaedtiaceae. Smith (1955) treats it as a Polypodioid genus on account of its terrestrial habit, erect rhizome, non-articulate stipes which resembles that of some species of *Polypodium*.

Copeland (1947) however on the basis of morphological characters, like the dictyostelic rhizome, dorsal sori and constant occurrence of perispores in the spores, places it among the Aspidiaceae. In addition to these characters we find some more Aspidioid features in *Tectaria amplifolia*. These are mentioned below.

*T. amplifolia* has in common with two other well-recognized Aspidioid ferns like *Peranema* and *Diacalpe* (Davie, 1912) the following features. The erect polystelic rhizome covered with branched fibrous roots, polystelic and long petiole with parenchymatous ground tissue, scales, superficial dorsal sori with stalked sporangia and large brown monolete spores with folded perispore or perine.

In the presence of unicellular hairs which are persistent on the main rachis and the rachis of the pinnae and pinnules, *T. amplifolia* resembles *Cystopteris* (Palser and Beric, *loc. cit.*) another member of the family Aspidiaceae.

The open dichotomous venation of *T. amplifolia* is met with in *Acrophorus* (Thomson Betty, 1943) another Aspidioid fern.

*T. amplifolia* in many of its characters like those of the rhizome which is covered by persistent leaf bases, paleae, and roots, dictyostelic vascular supply of the rhizome and stipe, their parenchymatous ground tissue, stomata restricted to the lower surface and undifferentiated mesophyll along with its spore characters resembles another Aspidioid fern *Metteuccia* (Nayar, 1961

and Nayar and Kazmi, 1963) In spore characters the spores of *T. amplifolia* resemble those of *Didymochena* (Stokey and Atkinson, 1954) and *Quercifilix* (Nayar, 1960), both members of Aspidiaceae

These facts support Copeland's reference of this genus to the Aspidiaceae at least so far as this species is concerned

### SUMMARY

The anatomy of the leptosporangiate fern *Tectaria amplifolia* (V A V R.) Christensen, one of the Aspidiaceae, has been worked out It shows a number of resemblances in its anatomy with two other well known Aspidioid genera like *Peranema* and *Diacalpe* In addition it shows a number of other Aspidioid features which strengthen its reference to this family

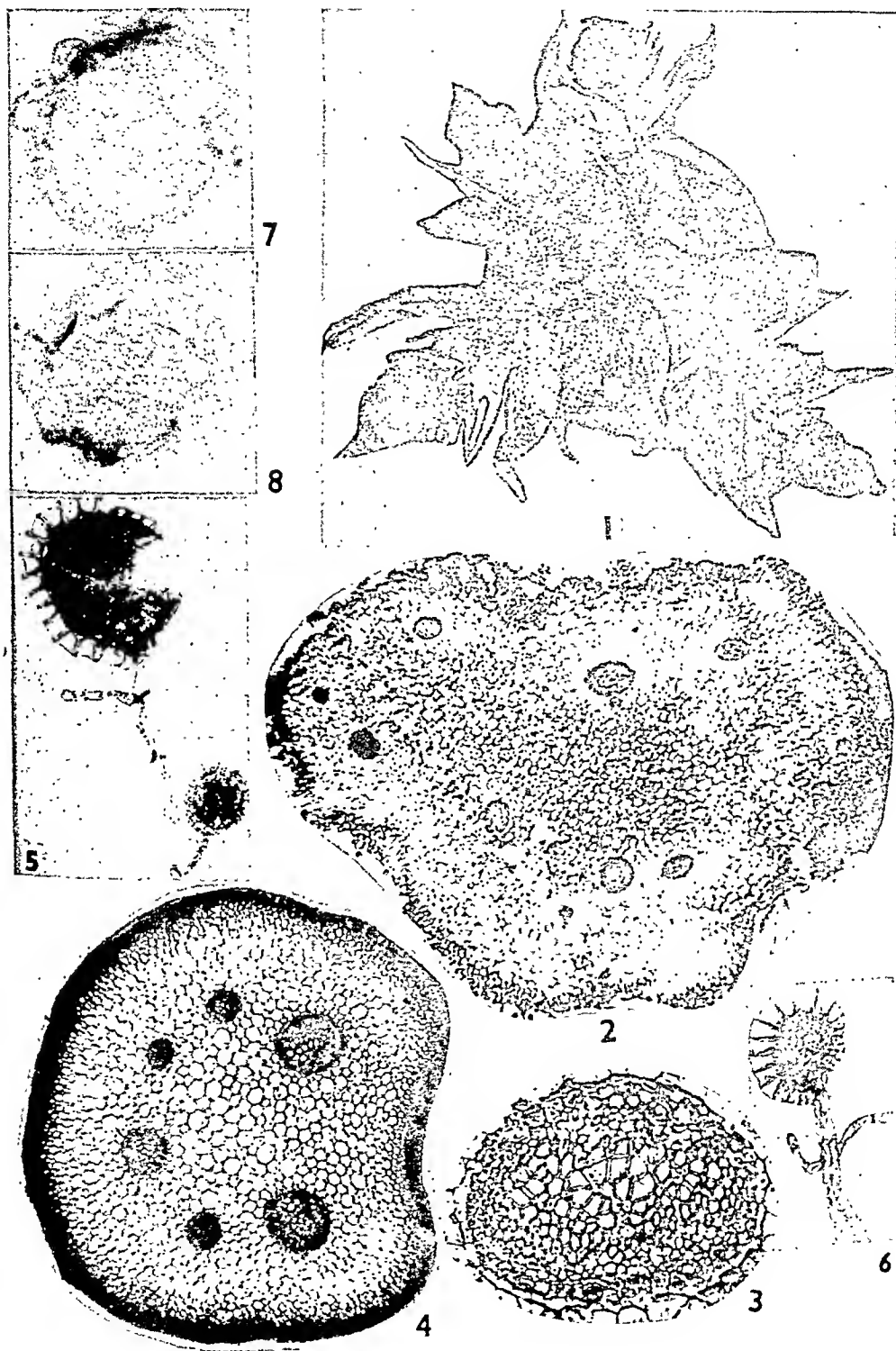
The rhizome is polystelic with 3-8 bundles, each bundle having exarch diarch xylem The main bundles of the rhizome give rise to the petiolar traces which number three The pinna traces are derived from these three bundles The abaxial superficial, indusiate sorus produces sporangia with incomplete annulus of 14-18 cells The spore output is 64 The spores are light brown in colour, bilateral and slightly planoconvex

### ACKNOWLEDGEMENT

We are very grateful to Dr B K Nayar who very kindly gave us many references to the literature on the subject

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FIGS. 1-8

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# EXPLANATION OF PLATE

- PHOTO. 1. Habit of the plant,  $\times 0.2$ .
- PHOTO. 2. T.S. of rhizome,  $\times 5.6$ .
- PHOTO. 3. Stele of the rhizome,  $\times 115$ .
- PHOTO. 4. T.S. of the petiole,  $\times 94$ .
- PHOTO. 5. Sporangium with one hair,  $\times 17$ .
- PHOTO. 6. Sporangium with two hairs,  $\times 17$ .
- PHOTO. 7. Spore—polar view,  $\times 765$ .
- PHOTO. 8. Spore—showing the ornamentation,  $\times 765$ .

# THE ANATOMY AND HISTOLOGY OF THE ALIMENTARY CANAL OF A HERBIVOROUS FISH *TILAPIA MOSSAMBICA* (PETERS)\*

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This work represents a part of a series of studies on the structure of the alimentary canal in relation to feeding habit of fishes *Tilapia mossambica* is an exotic cichlid fish which was imported to Madras from Ceylon in 1952. It has bred prolifically and is present in fresh and brackish waters in large numbers. It thrives well in sea-water also. This paper gives an account of its alimentary canal in relation to its herbivorous feeding habit. A similar account of an omnivorous fish *Mystus gulio* was previously given (Pasha, 1964).

The material was supplied by the fisheries station at Adyar. Standard fixatives and staining techniques were used throughout the investigation.

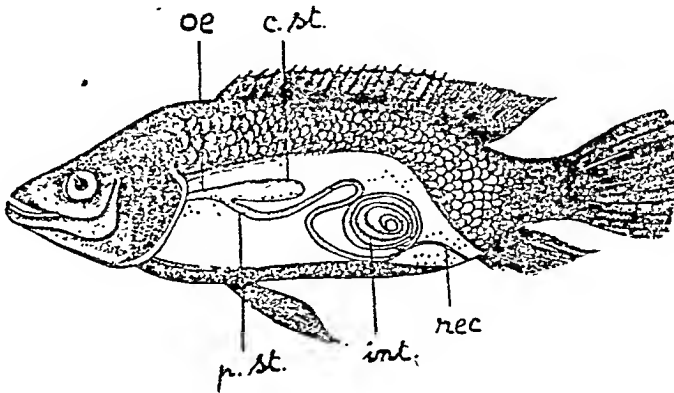
## FOOD, FEEDING HABITS AND GROSS ANATOMY

The stomach contents of about 25 fish were examined. The contents consisted chiefly of algae. There were a few higher aquatic plants. In the laboratory they fed on algae that were present on the stones and on the sides of the glass. Under starvation, they took rice and even bits of earthworm. According to Peter Devadas and Chacko (1953), *Tilapia mossambica* has a number of favourable qualities which make this fish ideally suited for culture in South India. One of the features they have reported on is that it is non-cannibalistic.

The alimentary canal of *Tilapia mossambica* consists of the mouth, buccal cavity, pharynx, oesophagus, stomach, intestine and rectum. Pyloric caeca are absent (Text-Fig. 1). The mouth is terminal. The gape of the mouth is about 1.7 cm. in a fish measuring 12 cm. The lips are thick. The lower jaw is a little longer, consequently the mouth opens rather upwards. The upper jaw is protrusible, when it is protruded the mouth becomes actually

\* Formed part of the thesis approved by the University of Madras for the M.Sc. degree

terminal. Both the jaws bear teeth. Each tooth has its projecting end slightly flattened and divided into two or three teeth, like those of a saw. In the upper jaw the teeth of the outermost row are longer and their position is such that when the upper jaw is protruded, the teeth are so thrust forward as to be used to scrape off the surface of stones, water plants, etc.



TEXT-FIG. 1

There is a rudimentary tongue which has its anterior end free and the posterior part attached to the floor of the buccal cavity. The floor of the cavity is flat and the roof is arched. The buccal cavity is broad anteriorly and narrows down posteriorly. The oral valves are present just behind the lips. The maxillary valve is broader. The mucous membrane lining the buccal cavity is thrown into folds, both longitudinally and transversely forming a network. It is always slimy due to copious secretion of mucus.

The pharynx, where the gills are attached, opens out through the gill slits. The gill rakers are reduced to small knob-like projections. Consequently, the fish cannot collect finer particles of food as a plankton feeder does. The mucous membrane covering the pharynx is a continuation of the buccal cavity but there are no folds visible to the naked eye. The pharynx bears dorsal and ventral teeth patches or apparatus which are bulged and cushion-like. The teeth of the pharyngeal patches are all alike. They are long with slightly curved pointed tips. The dorsal and ventral patches of teeth can be moved, the dorsal patches working against the ventral patches.

Das and Moritra (1955) are of opinion that gill rakers, pharyngeal and other teeth patches crush and masticate food in omnivorous and carnivorous fishes. According to Al-Hussani (1949) the relative development of the pharyngeal masticatory apparatus (horny pad, pharyngeal teeth) bears a

direct relationship to the amount of plant food in the diet. In *Tilapia mossambica*, probably the oral teeth are used for scraping the plant materials and the pharyngeal dentition which is well developed is used for grinding.

The pharynx leads into the oesophagus which is cylindrical and short, measuring only 0.8 cm in a fish 13 cm in length. The oesophagus has complex longitudinal folds which are continuous with those of the stomach. Posteriorly it leads into a bag like structure which is the corpus or body of the stomach. This serves as a reservoir for the ingested food. The shape of the stomach largely depends on the amount of food contained. The pyloric stomach starts as a small tube from the right side of the stomach very near the junction between the oesophagus and the body of the stomach. A pyloric constriction is present. The proximal part of the intestine is in the form of a U shaped loop, the space between the two limbs of the loop being occupied by a projecting lobe of the liver. The rest of the intestine is coiled in the form of a cork screw. The intestine is very long measuring 103 cm in a fish 13 cm long (Table I). It is about eight times as long as the fish. Great length of the intestine is usually a characteristic feature of herbivorous forms. An intestino rectal valve is not present. Therefore the posterior part of the intestine which has shallow mucosal folds may be considered as the rectum.

### HISTOLOGY

The lip is made up of mucosa and submucosa (Pl. XV, Fig. 1). The mucosa consists of stratified epithelium, basement membrane and a thin stratum compactum. The epithelium is thrown into very shallow folds and has many layers of cells. The cells have different shapes. They are polyhedral at the surface, columnar in the centre and of irregular shapes at the bottom. Many of the cells of the topmost layers have no distinct nuclei and some of these cells do not show a compact arrangement. Probably these are worn-out cells. There are numerous taste buds which are elongated and flask-shaped. They are found on the papillary projections of the submucosa. Each taste bud encloses a few elongated cells which stain deeply and have elongated processes forming a compact bundle. Presence of a large number of taste buds indicates that the fish has a well-developed gustatory faculty. Mucous cells are completely absent. The submucosa is made up of loose connective tissue which is areolar in nature.

The buccal wall is made up of mucosa, submucosa and muscularis. The mucosa consists of stratified epithelium, basement membrane and stratum

compactum. The mucosa of the roof of the buccal cavity is not uniform throughout. Anteriorly, it is more or less like that of the lips, though the folds are slightly deeper (Pl. XV, Fig. 2). It has a typical stratified epithelium like that of the lips. Mucous cells are absent though they are present in the floor. However, a little posteriorly the mucous membrane not only has more pronounced folds which are both transverse and longitudinal forming a network, but also changes from a typical stratified epithelium to one that is mostly composed of mucous cells (Pl. XV, Fig. 3). The mucous cells occurring posteriorly are mostly cylindrical and are arranged in rows. They rest on the basement membrane and extend up to the free surface of the epithelium. They stain characteristically with thionin. Taste-buds are present only in the anterior part of the buccal cavity. They resemble those of the lips. The basement membrane is thin, so also is the stratum compactum. The submucosa is in the form of a network as it is areolar in nature. The muscularis is found only in the floor of the buccal cavity and consists of inner circular and outer longitudinal fibres. All the fibres are striated.

There are two oral valves. The maxillary oral valve consists of mucosa dorsally and ventrally, made of stratified epithelium with submucosa between (Pl. XV, Fig. 4). The mucosa of the ventral side shows 6-8 layers of cells. There are mucous cells of spherical shape with basal nuclei. In addition to these mucous cells a few large spherical or elongated mucous cells with central nuclei are also present. Taste-buds are not numerous. The dorsal mucosa has the same structure as that of the ventral mucosa except that the taste-buds are very rare. The submucosa is made up of wavy fibres and shows large meshes. It is highly vascular. The mandibular valve resembles the maxillary valve, except that the dorsal mucosa shows numerous taste-buds which are borne by the papillary projections of the submucosa and that the mucous cells are also larger in number. A transverse mucosal fold just below the mandibular valve is rather broad and looks like another mandibular valve (Pl. XV, Fig. 5).

The pharynx is made up of mucosa, submucosa and muscularis (Pl. I, Fig. 6). Currey (1939) deals with the pharyngeal region of the Carp, *Cyprinus carpio communis*, elaborately dividing it into anterior and posterior regions on histological differences. In *Tilapia mossambica* the tunics are more uniform, there being only minor differences.

The mucosa is made up of stratified epithelium, basement membrane and stratum compactum which is thin. It is thrown into folds which are shallow in the anterior part of the pharynx and deep posteriorly. The stratified epithelium is not uniformly thick, and consists of from 10 to 20 layers



of cells. Superficial layers have polyhedral cells and in the base the cells are either oval or cylindrical. A large number of mucous cells occur in the epithelium. They are of two kinds. Those occurring superficially are spherical with darkly stained basal nuclei. The other kind occurs in the deeper layers of which some are cylindrical, some spherical and some oval (Pl XV, Fig 6). Usually, the spherical nucleus occurs in the centre and the contents appear in the form of a network. However, in some of them the nuclei are found shifted towards their bases and the cells appear empty, probably due to the mucus having been discharged. Further study of these cells may be of significance due to the fact that the fish is a mouth-breeder. Numerous taste buds occur, particularly in the posterior part of the pharynx. They resemble those of the lips except that they are larger. The exposed extremity is sunk in a pit which is probably similar to the gustatory pore seen in the mammals. The epithelium is supported below by a basement membrane. The stratum compactum found next to the basement membrane is very thick and wavy in outline. It consists of fibres arranged very closely. The submucosa is extensive and consists of areolar connective tissue having very large meshes. The muscularis is in two layers, an inner longitudinal and an outer circular. Both have striated fibres.

The proximal part of the oesophagus which lies outside the body cavity consists of four layers, mucosa, lamina propria, submucosa and muscularis. The distal part lying inside the body cavity has in addition a serous layer. There are 7-8 deep folds in the anterior and middle regions (Pl XV, Fig 7). Posteriorly, there are more folds which are mostly shallow.

The mucosa is made up of columnar cells, goblet cells and undifferentiated cells (Pl XV, Fig 8). There are also a few simple mucus-secreting cells. The columnar cells occur in one or two layers throughout the oesophagus. They are present in the edges of the folds, where they are slender, broad towards the lumen and narrow towards the base. They are also present on the sides and in the crypts where they are larger. The goblet cells are numerous occurring intermingled with simple mucus-secreting cells and columnar cells (Pl XV, Fig 8). The undifferentiated cells of the epithelium are present in the edges of the folds and at the base of the columnar and mucous cells. Many leucocytes which may have migrated are also found scattered in the epithelium. Taste buds are absent in the entire oesophagus. This feature has been noticed by Al-Hussaini (1945, 1946, 1947), Rogick (1931) and Sarbahi (1940) in the fishes studied by them.

The lamina propria is composed of dense areolar tissue which continues into the mucosal folds to the very tips. It is highly vascular. The sub-

mucosa resembles the lamina propria and it is difficult to distinguish them from each other but for the fact that in the meshes of the submucosa there are numerous scattered bundles of longitudinal muscle fibres. The circular and longitudinal layers form the muscularis. Both are of striated fibres. The serosa is very thin and is made up of flattened cells.

The stomach is of the caecal type. Corpus and pyloric parts are well differentiated with regard to histological details also. There are about 10–12 primary longitudinal folds. They are very thick and quite conspicuous. The mucosa is made up of two types of epithelium, superficial epithelium and glandular epithelium.

The superficial epithelium is composed of compactly arranged columnar cells (Pl. XV, Fig. 9). The cells lining the crypts are shorter and almost cylindrical. The nuclei are large and oval. They are situated in the basal half of the cells, all the nuclei occurring at the same level in the epithelium. About one-fourth of the cell nearer the lumen stains pale blue while the rest stains red and appears granular. With thionin the above-mentioned one-fourth of the cell stains red showing the presence of mucus. This is in agreement with Al-Hussaini's (1946) observation that the columnar cells have the function of mucus secretion.

The glandular epithelium consists of the gastric glands. These are all simple and tubular, made up of cells which are rhomboidal in shape with a spherical nucleus in each at the centre. They have a very narrow lumen. The zymogen granules present in these cells are distributed uniformly. There is no differentiation of the cells into oxyntic and peptic cells as all of them are similar. Each gland is surrounded by a thin layer of lamina propria which helps to keep the glands in position. The glands open in groups of two or three into the gastric crypts. There are no neck cells.

The pyloric stomach has 6–8 longitudinal folds which are branched (Pl. XV, Fig. 10). The epithelium of the mucosal folds resembles that of the corpus. There are no gastric glands.

The lamina propria consists of very loose connective tissue and it is similar throughout the stomach. The muscularis of the corpus has an inner circular and an outer longitudinal layer, both of which consist of unstriated fibres. In the pyloric stomach, the circular layer is mostly made of unstriated fibres. However, here a few striated fibres also occur probably extending from the oesophagus (Pl. XV, Fig. 11). There is plenty of connective tissue among the fibres. There are two longitudinal layers, one outer to the circular and another inner to it. Both of them are in

bundles The inner layer has a large number of bundles which are found in the meshes of the submucosa The serosa is thicker than that of the oesophagus The cells are not seen clearly

There is no special valve between the pyloric stomach and the intestine However, mucosal folds in the region of the pyloric constriction are very thick and deep They are directed towards the lumen of the pyloric stomach (Pl XV, Fig 12) Probably, they are responsible for regulating the passage of food from the stomach to the intestine

The intestine can be divided into an anterior part, a middle part and a posterior part or the rectum All these parts are fundamentally similar in structure and are made up of the same layers throughout, the differences being few and minor The folds of the duodenum are thin with pointed crests and broader bases They are mostly longitudinal in arrangement This condition is noticed in the middle intestine also (Pl XV, Fig 13) In the posterior region the folds are thick, shallow and fewer

The epithelium is made up of two principal types of cells, the columnar and the goblet cells The columnar cells are so numerous that the epithelium is mostly composed of these cells The goblet cells are few and scattered The columnar cells are typically cylindrical and high The nucleus is oval and shows a distinct chromatin network A thin sheet, which is striated, forms a top plate It is continuous over the columnar cells except in those regions where the goblet cells open The goblet cells have a swollen portion and a narrow tail portion The latter contains the nucleus (Pl XV, Fig 14)

The lamina propria is composed of richly vascular areolar connective tissue which merges with the submucosa The submucosa is also areolar in nature The muscularis consists of an outer longitudinal layer and an inner circular layer It is of uniform thickness Both the layers are made of unstriated fibres The serosa is made up of one or two layers of flattened cells There is a little subserous connective tissue between the serosa and muscularis

In the rectum the thickness of the musculature is almost the same as in the anterior and middle parts However, the rectal region can be easily distinguished by the shallowness and the greater thickness of the mucosal folds Further, the lamina propria occupies a wider area (Pl XV, Fig 15) The columnar cells and the goblet cells resemble those of the intestine

TABLE I

*Length measurements of various regions of the alimentary canal of Tilapia mossambica*

No.	Length of fish in cm.	Bucco- pharynx in cm.	Oesopha- gus in cm.	Stomach in cm.		Intes- tine in cm.
				Corpus Pyloric		
1	13.0	2.5	0.8	2.6	2.1	103.0
2	12.6	2.4	0.8	2.5	2.0	98.5
3	12.0	2.2	0.7	2.3	1.8	95.5
4	11.7	2.0	0.7	2.2	1.8	94.0
5	11.2	1.9	0.6	2.1	1.7	92.5

## SUMMARY

The anatomy and histology of the alimentary canal of a herbivorous fish has been described.

The mouth is terminal. The jaw teeth and pharyngeal teeth patches are well developed. The gill rakers are reduced into small knob-like projections.

Taste-buds are present in the lips, buccal cavity and pharynx. The pharynx has the largest number of taste-buds. The stomach is of 'caecal' type and the gastric glands are present only in the body of the stomach. There is no differentiation of glandular cells into oxyntic and peptic cells. The pyloric stomach has thicker musculature.

The intestine is very long. It is about eight times the length of the fish. The intestinal mucosa has only two kinds of cells, columnar and goblet cells.

An ileo-rectal valve is not present. The rectal musculature is not thicker than that of the intestine.

## ACKNOWLEDGEMENTS

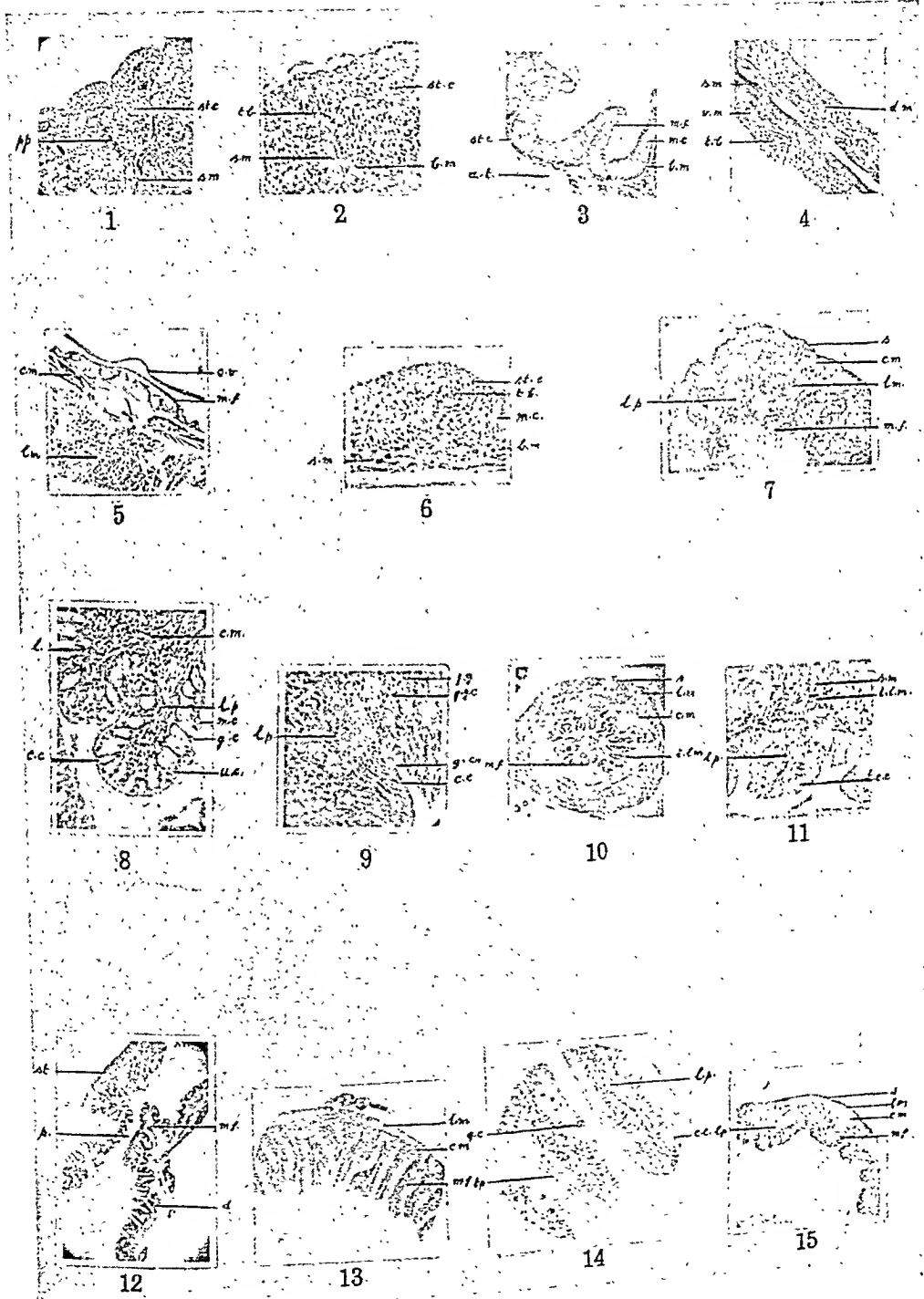
I am grateful to Prof. P. K. Menon, former Professor and Head of the Department of Zoology, Presidency College, Madras, for suggesting the problem and guidance.

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## EXPLANATION OF PLATE

- FIG 1 TS of lip
- FIG 2 TS of anterior buccal membrane
- FIG 3 TS of posterior buccal membrane
- FIG 4 TS of maxillary oral valve.
- FIG 5 TS of mandibular valve
- FIG 6 TS of pharynx.
- FIG 7 TS of Oesophagus.
- FIG 8 TS of Oesophagus (part enlarged)
- FIG 9 TS of stomach showing gastric glands.
- FIG 10 TS of pyloric stomach.
- FIG 11 TS of pyloric stomach (part enlarged)
- FIG 12 LS of pylorus.
- FIG 13 TS of intestine
- FIG 14 TS of intestine part enlarged.
- FIG 15 TS of rectum.



FIGS. 1-15

#### ABBREVIATIONS

*a.t.*, areolar tissue; *b.m.*, basement membrane; *c.c.*, columnar cell; *c.m.*, circular muscle layer; *c.st.*, corpus of stomach; *d.*, duodenum; *d.m.*, dorsal mucosa; *g.c.*, goblet cell; *g.cr.*, gastric crypt; *g.g.*, gastric gland; *g.g.c.*, gastric gland cell; *il.m.*, inner longitudinal muscle layer; *int.*, intestine; *l.*, leucocytes; *l.m.*, longitudinal muscle layer; *l.p.*, lamina propria; *m.c.*, mucous cell; *m.f.*, mucosal fold; *oe.*, oesophagus; *o.v.*, oral valve; *p.p.*, papillary projection; *rec.*, rectum; *p.st.*, pyloric stomach; *s.*, serosa; *s.m.*, submucosa; *st.*, stomach; *st.c.*, stratum compactum; *st.e.*, stratified epithelium; *t.b.*, taste-bud; *t.p.*, top plate; *u.c.*, undifferentiated cell; *v.m.*, ventral mucosa.

# A CONTRIBUTION TO THE STUDY OF ROOT NODULES IN SOME LEGUMES

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## INTRODUCTION

THIS paper deals with the root nodules and their developmental morphology in some legumes *Trigonello foenum-graecum* and *Canavalia gladiata* which are cultivated for vegetable and fodder, *Trigonella occulta* which is a weed found usually in wet places along the margins of ponds or tanks and *Phaseolus mungo* which is rotated with wheat

## MATERIAL AND METHODS

Plants of *Trigonello foenum graecum*, *T occulta*, *Canavalia gladiata* and *Phaseolus mungo* were raised in pots each with 1,000 gm of soil collected from a field in which a leguminous crop was raised previously. The soil moisture was maintained at 75 per cent of the water-holding capacity. Nodules of different ages of these plants were fixed in Navashin's fluid for 24 hours and rinsed with 70 per cent alcohol. They were embedded in paraffin and sectioned at 8 to 12 microns thick after customary dehydration and infiltration. The sections were stained with Heidenham's haematoxylin with erythrosine as counter-stain.

## OBSERVATIONS

*Trigonella foenum graecum* Linn and *T occulta* Delile

In *Trigonella foenum-graecum* the first nodule is observed on the primary root close to the surface of the soil on the ninth day of the seedling. This is followed by the appearance of more nodules on roots of various ages. The nodules are at first spherical, but later branch into a two-lobed or a fan-shaped structure within four to five days of their initiation. The fan shaped nodules are common on the primary and secondary roots close to the surface of the soil, their frequency decreases with depth, while the spherical and bilobed nodules are scattered all over the branches of the roots (Fig 1). In



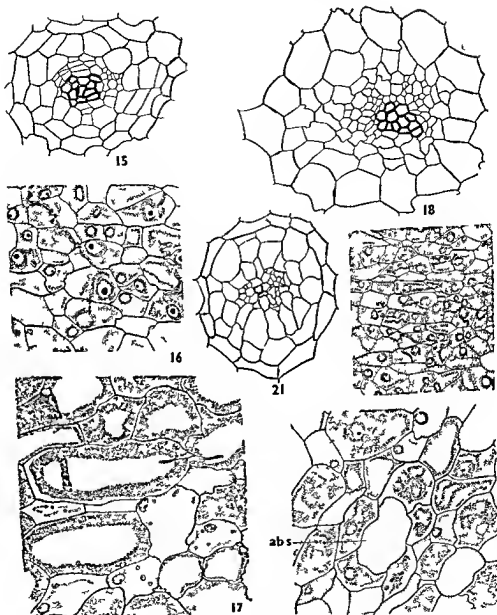
*T. occulta* the nodules are cylindrical or club-shaped and those which are present close to the surface of the soil are two-lobed (Fig. 4).

Generally in *T. foenum-graecum* most of the nodules are pink and effective. However, in *T. occulta* the large, cylindrical nodules are pink and effective and the small, globular nodules which are colourless or dark brown are ineffective and their frequency of occurrence is low.

In both the species of *Trigonella* investigated infection of the root is through root-hairs. The infected root-hair neither enlarges nor thickens, but the hypodermal cortical cell distends to twice its original size. The cortical cells through which the main infection thread travels are also large. The funnel-shaped expansions of the infection thread, close to the wall, appear prominent in the enlarged cells (Figs. 16, 17). The infection thread also shows affinity with the nucleus of the host cell, moves towards it and may even press against the same. There seems to be some correlation between the thickness of root cortex and the depth of penetration of the infection thread. In the thick six-layered cortex of the primary root the infection thread penetrates half-way down, while in the thin, four-layered cortex of the secondary and tertiary root, the infection thread traverses the entire cortex up to the endodermis but does not enter the stele. In both the species of *Trigonella* the nodule is exogenous in origin.

The mature nodule is differentiated into four characteristic regions: nodule meristem, nodule cortex, vascular system and bacteroid region (Figs. 2, 5). The nodule meristem is conspicuous at the distal end and is composed of thin-walled polygonal cells with dense contents and a conspicuous nucleus. However, cessation of meristematic activity of certain groups of cells restricting the same to certain others, soon initiates branching of the nodule. The cells which are newly produced posteriorly by it, enlarge and become parenchymatous. Some of these are invaded by the infection threads which will, thus, move towards the apex as the nodule grows. The infection thread always shows affinity with the host nucleus and move towards the latter during its intracellular course (Fig. 16).

The nodule cortex is homogeneous and parenchymatous. The vascular supply of the nodule begins as procambial strand formed by the divisions of the cortical cells situated between the stele and the region of infection of the root cortex. This soon establishes connection with the protoxylem point of the root and progresses towards the distal end of the nodule. In *T. foenum-graecum* four such vascular connections are established with the root stele, two of which supply each side of the nodule. These vascular



FIGS. 15-21. Figs. 15-17 *Trigonella foen-graecum*. Figs. 18-20 *Canavalia gladiata*. Fig. 21 *Phaseolus mungo*. Fig. 15 Vascular bundle with phloem on three sides of xylem  $\times 340$ . Fig. 16 Enlargement of a portion of newly formed cells being invaded by infection threads  $\times 500$ . Fig. 17 Magnified view of a few infected and uninfected cells see the funnel shaped swelling of the infection thread close to cell wall  $\times 500$ . Fig. 18 Vascular bundle with phloem on three sides of xylem  $\times 340$ . Fig. 19 Newly formed cells being invaded by infection threads  $\times 500$ . Fig. 20 Enlarged region of bacteroid tissue showing absorption spots,  $\times 500$ . Fig. 21 Bicollateral bundle  $\times 340$  (abs absorption spots).

strands of the nodule branch repeatedly during their upward course and extend along the inner cortex of the nodule. They are separated from the bacteroid zone by a parenchymatous layer two to three cells thick. The vascular strands fail to anastomose at the apex of the nodule because of the distal nodule meristem. 22 to 24 vascular bundles surround the bacteroid zone in cross-section of the fan-shaped nodule in *T. foenum-graecum* and are collateral throughout their course (Fig. 15).

The bacteroid tissue increases in quantity by fresh invasion of new cells produced by the meristem. In *T. foenum-graecum* and *T. occulta* 72 and 86 per cent. of the bacteroid tissue are infected. These infected cells are twice the size of uninfected cells (Figs. 3, 17). Numerous vacuoles of different sizes appear around the nucleus. Later, they merge together into a large central vacuole of half the volume of the cell pushing the nucleus to one side in *Trigonella*. The nucleus of the infected cell distends, becomes amoeboidal (Figs. 3, 17). However, the uninfected cells possess thin cytoplasm, small nuclei and abundant starch grains.

With the increasing age the pink nodules turn dark brown. This change in colour is accompanied by other changes such as wrinkling of the surface and loss of turgidity. The bacteria become clumped at the centre and less stainable. The degeneration of nodule proceeds from the base upwards.

The ineffective nodule in *T. occulta* (Fig. 6) has less activity of nodule meristem. The nodule cortex consists of two to four layers of large cells. The vascular supply is ill-developed. Only 30 to 40 per cent. of the cells of the central region are large and they exhibit disintegration of their meagre bacterial content. The infection threads run parallel, invading the new cells formed by the distal nodule meristem.

#### *Canavalia gladiata* (Jacq.) DC.

In *Canavalia gladiata* the first nodule appears on the secondary root on the sixth day of the seedling when the first true leaves unfold and subsequent nodule formation is on the secondary, tertiary and quaternary roots and never on the primary root (Fig. 7). The nodule is spherical to begin with and becomes cylindrical or two-lobed (Fig. 9) in the manner described for *Trigonella*. Both effective and ineffective nodules are formed. Observations made on the mean number of nodules per plant, when one, two or three plants are grown per pot, show that the number of nodules per plant is highest when there is only one plant per pot. The number of nodules per plant decreases with increasing number of plants per pot. In all cases the mean

number of nodules per plant per pot is maximum on the 29th day and decreases thereafter. The size of the nodules increases with age.

Entry of the rhizobia is through root hairs. The infected root hair enlarges four times its original size. The infection thread is single and branched in the root hair itself (Fig 9) and remains conspicuous at all stages of development of the nodule.

The mature nodule of *Canavalia gladiata* has a nodule meristem, a nodule cortex, vascular supply and a bacteroid zone (Fig 9). The nodule meristem is prominent at the distal end in the cylindrical or lobed nodules. The cells which are produced on the lower side by the distal meristem are progressively invaded by the infection threads whose size is uniform in the young infected cells (Fig 19). However, funnel shaped expansions of the infection thread close to the host cell wall become obvious in the enlarged cells. The nodule cortex is heterogeneous, with the middle sclerenchymatous layers being sandwiched by parenchyma.

The vascular supply in *Canavalia* starts as a procambial strand between the stele and the point of infection in the root cortex, subsequently, it establishes connection with the protoxylem point of the host root. It consists of one or two traces. The vascular strands that progress to the distal end of the nodule branch and surround the bacteroid zone but do not anastomose at the apex because of the distal nodular meristem. The number of vascular bundles that surround the bacteroid zone in cross section is 10 to 12. The bundles are amphicribal at the base and amphicribal or inversely collateral with phloem on three sides higher up (Fig 18).

The increase in the bacteroid tissue is brought about by the invasion of new cells produced proximally by the nodule meristem. 49 per cent of the bacteroid tissue are infected, they are nearly one and a half times larger than the uninfected cells.

During nodule decadence, the bacteria clump together and lose stainability. Most of the cells in the bacteroid zone exhibit disintegration and absorption spots (Fig 20). The degeneration of the nodule progresses from the base upwards, ultimately resulting in an empty shell whose wall is composed of the cortex traversed by the vascular bundles.

Two types of ineffective nodules occur. (1) large, slightly pink nodules, and (2) small, colourless ones. The larger ineffective nodules have diffused nodule meristem peripheral to the bacteroid tissue, heterogeneous cortex and well-developed vascular bundles. However, the infected cells are not

enlarged. The parenchymatous cells that surround the bacteroid tissue are four times larger than the infected cells and contain small nuclei and abundant starch grains. The small, colourless nodule is undifferentiated. The infected cells are indistinct with meagre bacterial content. The cortex is parenchymatous (Fig. 10).

The false or atypical nodules are also frequent in the form of collar-like swellings at the base of the lateral rootlet. They are the consequence of meristematic activities of the outer cortical cells.

### *Phaseolus mungo* Linn.

In *Phaseolus mungo* the first nodule appears on the primary root on the third day of the seedling when the first true leaves unfold. Subsequently, more nodules develop principally on the primary root in so close a succession that they collectively present the appearance of a bunch of grapes (Fig. 11); nodules also develop on secondary roots. The nodules are usually spherical. When the plants are 32 days old, the nodules situated close to the surface of the soil on the tap root decrease in number due to degeneration, while their number increases on the secondary and tertiary roots. The larger nodules are fewer than the smaller nodules. All nodules are pink and effective, but become brown with age.

Infection is through root-hairs. The infected root-hair itself enlarges four times its original size. The infection threads are in clusters of two or three and remain conspicuous at all stages of development of the nodule. A nodule may also be initiated by more than one infected root-hair (Fig. 12,  $rh_1$  and  $rh_2$ ). The infection thread penetrates the root cortex up to the middle, irrespective of the thickness of the cortex. The nodule is exogenous in origin.

The mature nodule is differentiated into nodule meristem, nodule cortex, vascular system and bacteroid region (Fig. 13). The meristem is inconspicuous and diffused, peripheral to the bacteroid region. The spread of the rhizobia in the bacteroid region is by the invasion of cells and not by the divisions of the infected cells as shown by the presence of infection threads in every infected cell. The nodule cortex is heterogeneous with the middle sclerenchymatous layers sandwiched by parenchyma on either side (Fig. 13).

The vascular supply of the nodule originates as a procambial strand by the division of the cortical cells situated between the stele and the infected part of the root cortex. This strand establishes connection with the protoxylem point of the root, progresses towards the distal end of the nodule,

TABLE I

*A brief resume of the present investigation*

	<i>Trigonella foenum graecum</i>	<i>T. occulta</i>	<i>Canavalia gladiata</i>	<i>Phaseolus mungo</i>
1 Appearance of first nodule	ninth day of the seedling		sixth day of the seedling	third day of the seedling
2 Position of nodules	primary root and roots of other orders	primary root and roots of other orders	secondary root and roots of other orders	primary root and roots of other orders
3 Portal of entry of root by the infection thread	root hair	root hair	root-hair	root-hair
4 Infection thread (i) number (ii) behaviour in the root hair	one unbranched	one unbranched	one branched	two to three unbranched
5 Mature nodule (i) meristem (ii) cortex (iii) vascular system	distal homogeneous four strands collateral throughout 22 to 24 vascular bundles surround the bacteroid zone do not anastomose distally	distal parenchymatous	distal heterogeneous, wiehing the one or two strands amphicribal at the base and amphicribal or inversely collateral higher up 10 to 12 vascular bundles surround the bacteroid zone	diffused and peripheral parenchyma and sclerenchyma one strand amphicribal at the base and amphicribal or bicollateral higher up 7 to 9 vascular bundles surround the bacteroid zone anastomose distally
6 Percentage of infected cells	72	86	49	70
7 Ineffective nodule present	present	present	present	not present
8 Atypical nodule	not present	not present	present	not present

branches around the bacteroid zone and ultimately anastomoses at the distal end of the nodule.

The number of vascular bundles that surround the bacteroid zone in cross-section is 7 to 9. Each bundle is amphicribal at the base and amphicribal or bicollateral at the distal region (Fig. 21). 70 per cent. of the cells of the bacteroid tissue are infected and are slightly larger than uninfected cells. Numerous vacuoles occur near the nucleus. In old nodules the bacteria are clumped together and take little stain (Fig. 14). The degeneration of the nodule proceeds from the centre to the periphery.

### DISCUSSION

From the present investigation it is clear that the appearance of the first nodule synchronises with the unfolding of the true leaves. Thornton (1929) also observed such a correlation in *Medicago sativa*. However, Bieberdorf (1938) failed to observe a correlation between the unfolding of the true leaves and the appearance of the first nodule in *Glycine max*. Thornton (1929) suggested that nodule initiation is controlled by the entire vegetative top; however, further studies are needed to elucidate this.

Generally, the single infection thread is present in the root-hair branches when it enters the cortex. This condition is seen in *Trigonella foenum-graecum*. In *Canavalia gladiata* the single infection thread branches in the root-hair itself. A similar case was reported by Thornton (1930) in *Medicago sativa*. The several infection threads seen in the root-hair of *Phaseolus mungo* are somewhat similar to what has been recorded in *P. vulgaris* by McCoy (1929). Further, in *P. mungo* nodule formation by two infected root-hairs has also been seen in the present work. Similar cases have been reported by Thornton (1930) in *Medicago sativa* and by Arora (1956) in *Cicer arietinum*.

The depth of penetration of the root by the infection thread varies in different plants. In *Trigonella foenum-graecum* and *Canavalia gladiata* studied by us and in *Glycine max* (Bieberdorf, 1938), the infection thread penetrates the thick tap root down to the middle of the cortex and the thin secondary roots down to the endodermis. However, in *Cicer arietinum* the situation is just the opposite (Arora, 1956 c). In *Phaseolus mungo*, as shown by the present study, the infection thread penetrates up to the middle of the cortex irrespective of the thickness of the cortex in the primary and the secondary roots. Thus it is clear that the thickness of the root cortex does not determine the extent of penetration of the infection thread. Since nodule initiation is known to be linked up with polyploidy of some host cells,

a study on the distribution of ploidy in the cells of the root cortex might throw light on the differences in the depth of penetration of the infection thread in different host plants

As regards the funnel shaped swelling of the infection thread close to the host cell wall our observations on the uniform diameter of the infection thread in the young infected cells, the appearance of funnel-shaped swelling close to the wall in the enlarged infected cells and the rupture of the infection thread in the middle, strongly suggest emaciation of the infection thread in the middle as a consequence of stretching during enlargement of the infected cell leaving behind the infection thread of normal size as a funnel-shaped swelling close to the cell wall

The spread of rhizobium in the nodules of *Trigonella foenum graecum*, *T. occultum* and *Conovolio gladiota* is by the invasion of new cells by the infection threads. This study has also shown that in *Phaseolus mungo*, in which there is diffused meristem at the periphery of the bacteroid zone, the spread of the rhizobium is by the invasion of the newly-formed cells and not by the division of the infected cells as in *P. vulgaris* (McCoy, 1929). Branched infection threads seen in the host cells in *P. mungo* confirm this

### SUMMARY

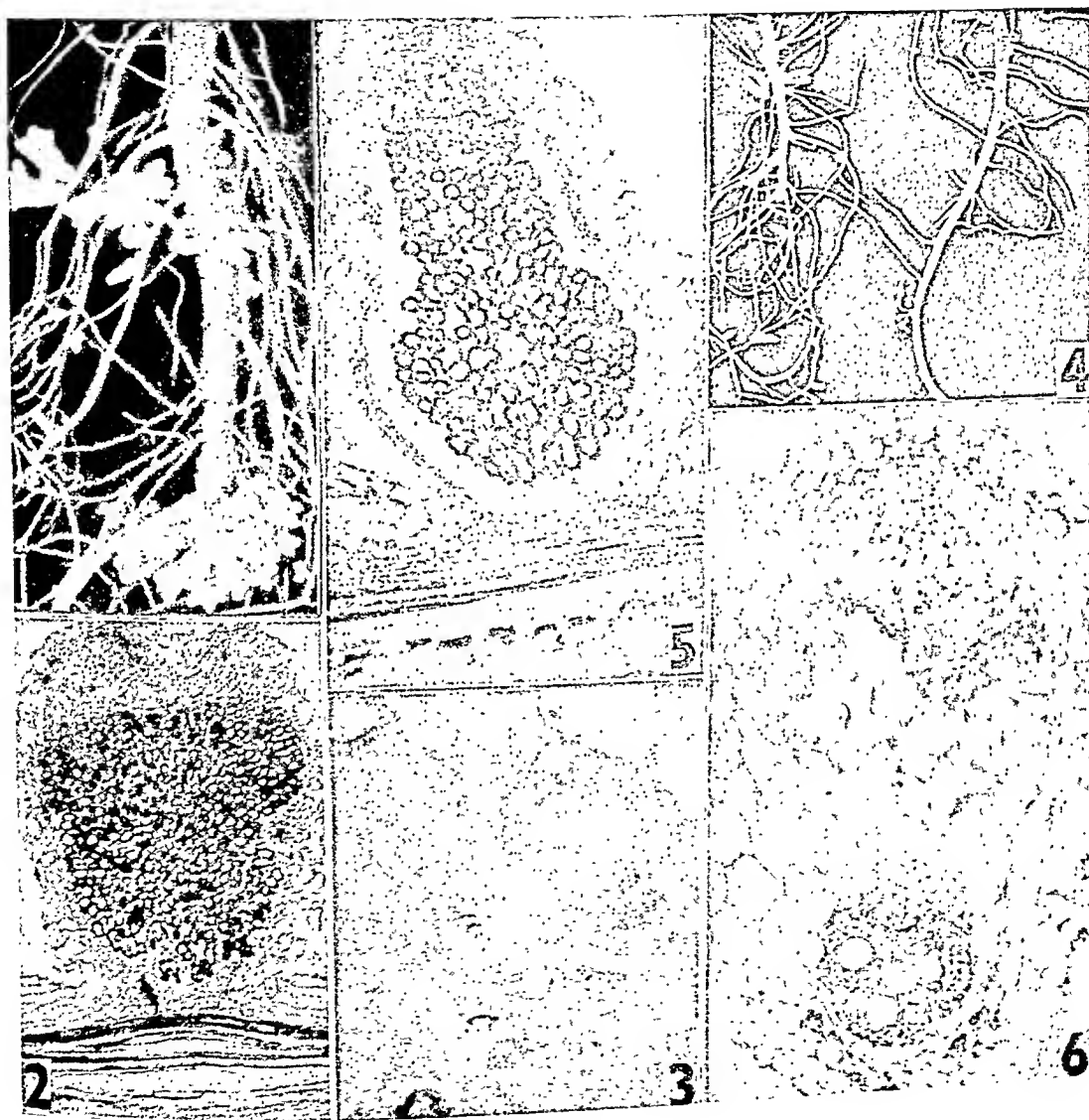
The structure and development of the root nodules of *Trigonella foenum-graecum*, *T. occultum*, *Conovolio gladiota* and *Phaseolus mungo* have been studied. A brief resume of the salient features of root nodules of these host plants is given in the form of a table and some of the points such as the initiation of nodule, the behaviour, the depth of penetration, and the spread of the rhizobium within host cells are discussed. The funnel shaped swellings of infection threads are considered to arise by emaciation due to stretching of the thread during the enlargement of the host cell harbouring it.

We are deeply indebted to Professor C V Subramanian, Head of the Department of Botany, for suggesting the problem, valuable suggestions and keen interest throughout this investigation.

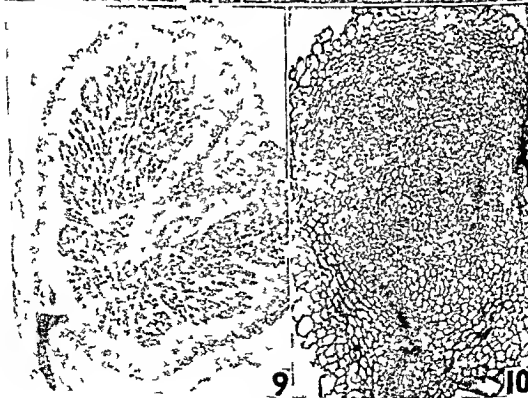
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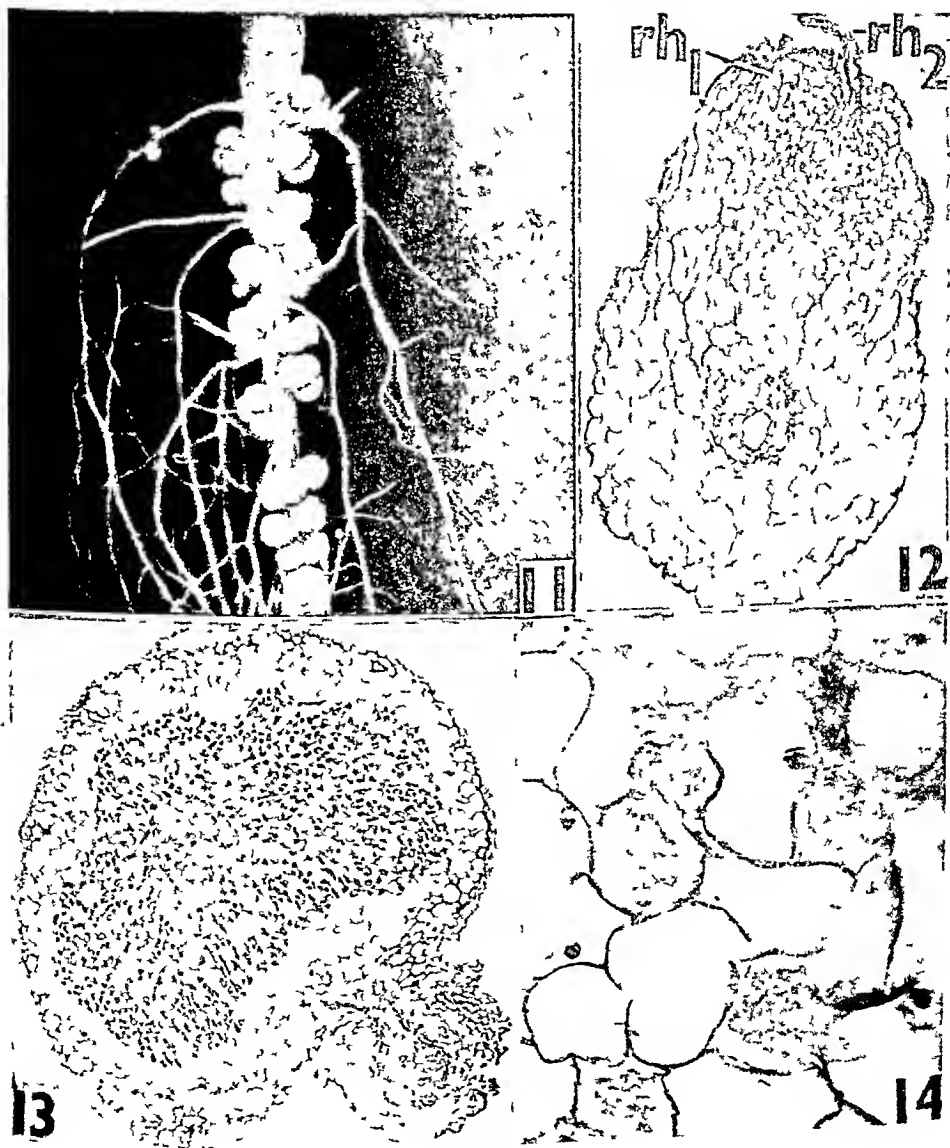
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FIGS. 1-6





FIGS. 11-14

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## EXPLANATION OF PLATES

### PLATE XVI

FIGS. 1-3. *Trigonella foenum-graecum*. FIGS. 4-6. *T. occulta*.

- FIG. 1. Habit of root nodule,  $\times 2$ .
- FIG. 2. Radial L.S. bilobed nodule with bacteroid tissue,  $\times 33$ .
- FIG. 3. Infected and uninfected cells, see the hypertrophied nucleus bordering the vacuole of infected cells and starch grains in the uninfected cells,  $\times 500$ .
- FIG. 4. Nodules on the primary and secondary roots,  $\times$  reduced  $2/3$ .
- FIG. 5. Radial L.S. effective nodule showing two vascular supplies,  $\times 89$ .
- FIG. 6. Ineffective nodule,  $\times 133$ .

### PLATE XVII

FIGS. 7-10. *Canavalia gladiata*.

- FIG. 7. Root bearing nodules,  $\times$  natural size.
- FIG. 8. Root-hair with branched infection thread,  $\times 1,000$ .
- FIG. 9. Bilobed effective nodule,  $\times 33$ .
- FIG. 10. Ineffective nodule,  $\times 133$ .

### PLATE XVIII

FIGS. 11-14. *Phaseolus mungo*.

- FIG. 11. Tap root with effective nodules,  $\times$  reduced  $2/3$ .
- FIG. 12. Nodule with root regions of infection,  $\times 153$ .
- FIG. 13. Mature nodule,  $\times 33$ .
- FIG. 14. Degenerating bacteroid tissue,  $\times 500$ .  
( $rh_1$  and  $rh_2$ , infected root-hairs,)

# MORPHOLOGICAL AND ONTOGENETIC STUDIES IN *SARCANDRA IRVINGBAILEYI* SWAMY

## III. The Stomata

BY M V RAMJI

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(Communicated by Professor T S Sadasivan, F A S C)

## INTRODUCTION

THE present investigation relates to studies on stomatal development in *Sarcandra irvingbaileyi* Swamy, a vesselless plant of the ranalean complex. Information on the stomata of Ranales is meagre when compared to studies on the anatomy of wood. From an ontogenetic point of view, the stomata in the angiosperms as a whole and ranalean complex in particular need more detailed study before any attempt can be made to make use of this structure on problems relating to phylogeny.

## MATERIALS AND METHODS

Source of materials was the same as described in an earlier publication (Ramji, 1961). Epidermal peels were either stained as per Foster's schedule (1934) or in acetocarmine and phloxine for a study of the distribution of stomata. Cleared whole mounts of leaves were also of immense value for following the stomatal types. Paradermal and transections were made at  $5\mu$  thicknesses and stained with hematoxylin and erythrosin, safranin and fast green combinations.

## OBSERVATIONS

In *Sarcandra*, the stomata are confined to the abaxial surface of the leaf. They are few on the midrib and along the lateral veins. The mature stomata are found mixed with the differentiating ones thus showing that their differentiation is spread over a long period. They are absent in a few cell layers (four to six layers) along the edges of the leaf. They are also absent in embryonic leaves of approximately 3 mm in length.

The stomata develop from actively dividing more or less polygonal cells of the abaxial surface (Fig. 1). The initial of the guard cell mother cell (*Gmc*)

shows greater avidity for nuclear and cytoplasmic stains. Its nucleus is larger and occupies a greater area of the cell (Fig. 2). This cell is lenticular or quadrangular in outline as seen in epidermal peels and paradermal sections (Figs. 1-3). To start with the walls of the *Gmc* appear straight (Fig. 3) but later assume a convex shape (Figs. 1, 2). It divides into two approximately equal halves (Figs. 3-5) and these later become the guard cells. Following division in the *Gmc*, the convexity of the walls and stainability of the cell contents are less pronounced. Following division, there is a heavy deposition of pectic substances in the central region of the separating wall (arrows in Fig. 7). This is the region where the walls of the guard cells separate to form the pore (Fig. 8). The intercellular substance swells in the central region of the cross-wall before the connection between the walls is weakened and the pore is formed.

The subsidiary cells are derived from the neighbouring epidermal cells and are not related to the *Gmc* ontogenetically. Thus, the stomatal type in *Sarcandra irvingbaileyi* may be classified as haplocheilic\* (see Esau, 1960, p. 345). It may be pointed out here that in a majority of instances the subsidiary cells are differentiated before division occurs in the *Gmc* (S in Figs. 2-4). Two or more subsidiary cells are organised around a *Gmc*. While in Fig. 2 two subsidiary cells flank along the long axis of the *Gmc*, in Fig. 4 one cell at the bottom and another cell on the side flank the dividing mother cell. The number of subsidiary cells surrounding a stoma varies on the same surface of the leaf. The common condition is for a subsidiary cell adjoining the longer axis of the guard cell to lie more or less parallel to the subsidiary cell on the other side (Fig. 6, B). This figure also shows other variations in the arrangement of the subsidiary cells. In one of these, the stoma is surrounded by four subsidiary cells (Fig. 6, F, G).

Usually, the division in the epidermal cell adjoining the *Gmc* results in a smaller subsidiary cell flanking the *Gmc* (S in Figs. 2 and 4). When these subsidiary cells are delimited, the side walls of the *Gmc* are prominently convex (Figs. 2, 3 and 4).

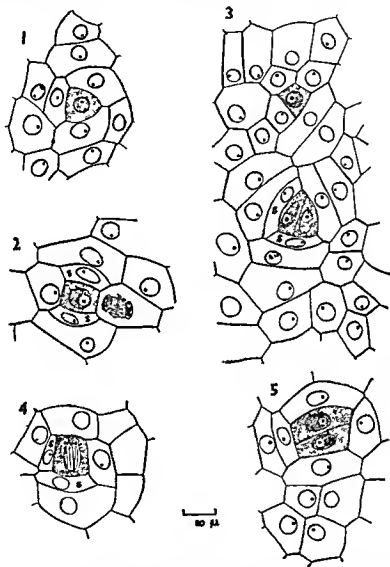
The stomatal type in the leaves of *Sarcandra* may broadly be classified as *paracytic* (Fig. 6, A, B).

#### DISCUSSION

The presence of mature stomata in the leaves of *Sarcandra* along with developing ones on the same surface shows that as in other angiosperms,

\* In fact the two types 'haplocheilic' and 'syndetocheilic' were proposed with reference to the gymnospermous stomata only but have found application to stomatal types met with in dicotyledons.

the ontogeny is spread over a considerable period. The protodermal cells of the abaxial surface divide many times before they give rise to the *Gmc*. Since the differentiation of the stomata occurs in a 'mosaic' fashion, it is not possible to pin down mother cells during early stages. A protodermal



FIGS. 1-5 Fig. 1 A lenticular guard cell mother cell (*Gmc*) in paradermal plane Fig. 2 A rectangular *Gmc* flanked by subsidiary cells (*S*) Fig. 3 Upper half of figure shows a *Gmc*, in the lower half is shown a lenticular mother cell that has divided into two guard cells flanked by subsidiary cells (*S*) Fig. 4 Division in a *Gmc*. The dividing *Gmc* is flanked by subsidiary cells (*S*) Fig. 5 Division in a quadrangular mother cell to form a pair of guard cells.

cell can be demarcated as *Gmc* only when it shows a specific or pronounced affinity for nuclear and cytoplasmic stains. This cell is lenticular or rectangular with slightly convex walls.

In *Sarcaandra*, the subsidiary cells are usually demarcated before a *Gmc* undergoes division. However, their number varies from one to three. These cells result from an unequal division of the neighbouring epidermal cells. The smaller cell of such a division is the subsidiary cell.

In the ranalean complex, investigations on stomatal ontogeny have been on *Drimys*, *Trochodendron*, *Tetracentron* (Bondeson, 1952), *Schisandra grandiflora* (Jalan, 1962), *Michelia champaka* and *Magnolia stellata* (Paliwal and Bhandari, 1962). Rao (1939) basing his observations on mature stomatal types regarded the stomata in certain Magnoliaceae syndetocheilic. Recently, ontogenetic studies have revealed their haplocheilic nature (Jalan, 1962); or, they may be variable (Paliwal and Bhandari, 1962). Thus both haplocheilic and syndetocheilic types occur in the Ranales. Among the vesselless dicotyledons, *Drimys* shows syndetocheilic type while *Sarcaandra* shows haplocheilic type. *Trochodendron* and *Tetracentron* exhibit an intermediate condition where the protodermal cell cuts off a subsidiary cell and a guard cell mother cell. The latter divides into two guard cells while the other epidermal cells become the subsidiary cells (Bondeson, 1952). Hence, the type of stomata (haplo- or syndetocheilic) when read together with the vesselless character affords no definite proof for arriving at any phylogenetic conclusion. It is seen that both types occur in the gymnosperms (Florin, 1931; Maheshwari and Vasil, 1961).

#### ACKNOWLEDGEMENTS

I am grateful to Professor T. S. Sadasivan for encouragement, lively discussion and facilities and Professor P. Maheshwari for suggestions and critical review of the manuscript. I am thankful to the National Institute of Sciences of India for the award of a junior fellowship during the tenure of which this work was completed.

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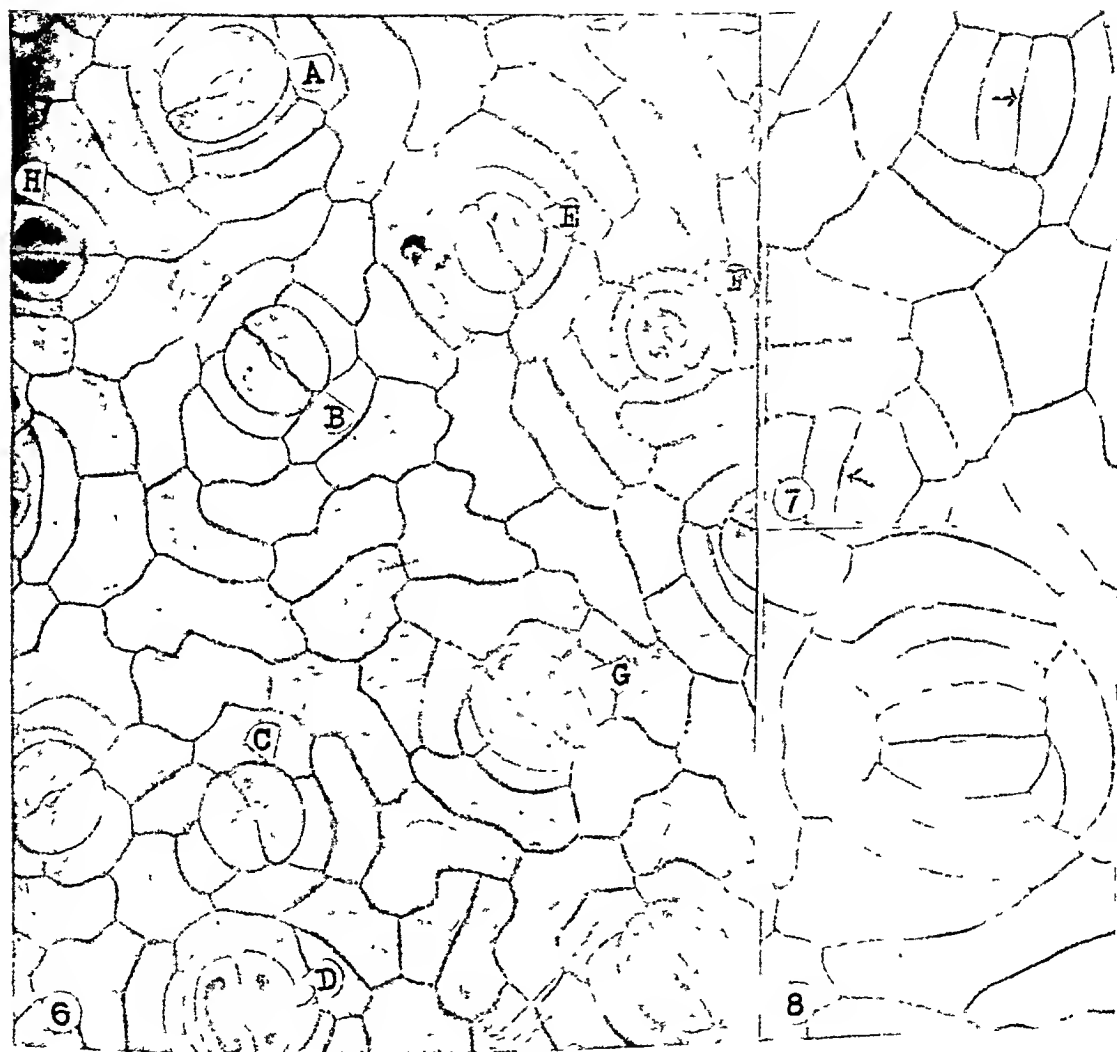
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### EXPLANATION OF PLATE XIX

- FIG 6 Photomicrograph of a portion of an epidermal peel showing various types of stomata; A and D, a pair of subsidiary cells face one cell on the other side of the stomata. B, the usual type of stomata, a subsidiary cell faces the one on the other side. C, stoma with one subsidiary cell. E, F, H, stomata surrounded by 3-4 subsidiary cells. G stoma with a pair of subsidiary cells, each subsidiary cell is transversely divided,  $\times 430$ .
- FIG 7 Portion of epidermal peel showing deposition of pectic substances in the centre, arrow indicates region of deposition  $\times 630$
- FIG 8 Portion of epidermal peel showing a stoma with a pair of subsidiary cells flanking along its longer axis, the central slit indicates separation of the walls to form the pore,  $\times 500$ ,



FIGS. 6-8

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